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# CYTOCHROMES AND PRENYLQUINONES IN PREPARATIONS OF CYTOPLASMIC AND THYLAKOID MEMBRANES FROM THE CYANOBACTERIUM (BLUE-GREEN ALGA) *ANACYSTIS NIDULANS*

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**The cytochrome and prenylquinone compositions were compared for cytoplasmic membranes and thylakoid membranes from the cyanobacterium (blue-green alga) *Anacystis nidulans*. Reduced-minus-oxidized difference absorption spectra at −196°C indicated that the thylakoid membranes contained photosynthetic cytochromes such as cytochrome *f*, cytochrome *b*-559 and cytochrome $b_6$, while cytochromes *c*-549 and *c*-552 were detected spectrophotometrically only after their release by sonic oscillation. The cytoplasmic membrane preparation contained one or two low-potential cytochrome(s) with α-band maxima at 553 and 559 nm at −196°C, which differed from the cytochromes in the thylakoid membranes. A cytochrome specific to the cytoplasmic membranes was also found by heme-staining after lithium dodecyl sulfate-polyacrylamide gel electrophoresis. Both types of membranes contained the three prenylquinones plastoquinone-9, phylloquinone and 5′-monohydroxyphylloquinone, but in different proportions.**

## Introduction

Cyanobacteria can perform oxygenic photosynthesis in light and respire in the dark [1–3]. However, the interaction between the photosynthetic and respiratory electron-transport systems is still in question. Some investigators suggest that both electron-transport pathways share certain components within the thylakoid membranes [4–7]. Lockau and Pfeffer [8,9] did not detect cytochromes in their cytoplasmic membrane preparation from *Anabaena variabilis* and inferred that there is no electron transport system in the cytoplasmic membranes. Some other investigators claim that the cytoplasmic membranes are a site of respiration [10,11].

We have isolated the cell envelope and cytoplasmic membranes from *A. nidulans* and studied their biochemical characteristics [12,13]. Since chlorophyll *a* is localized only in the thylakoid membranes, we concluded that photosynthetic electron-transport reactions occur only in these membranes. The next question is whether the cytoplasmic membranes of *A. nidulans* have an elec-

Abbreviations: LDS, lithium dodecyl sulfate; HPLC, high-performance liquid chromatography; TMBZ, 3,3′,5,5′-tetramethylbenzidine; TLC, thin-layer chromatography; Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulphonic acid.

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tron-transport system. The present study showed that cytoplasmic membranes contain cytochromes which differ from those in the thylakoid membranes and a considerable amount of plastoquinone-9, indicating that they do have their own electron-transport system.

## Materials and Methods

*A. nidulans* was obtained from the Algal Collection in the Institute of Applied Microbiology, University of Tokyo, and was grown photoautotrophically at 28°C as described previously [13].

Cytoplasmic membranes and thylakoid membranes were prepared from the cells as described previously [13]. Cells amounting to 5 ml packed cell volume were suspended in 50 ml of 600 mM sucrose/2 mM EDTA/5 mM Tes-NaOH buffer (pH 7.0), and incubated with 10 mg lysozyme (final 0.02%) at 30°C for 2 h. The lysozyme-treated cells were collected by centrifugation at $3500 \times g$ for 5 min, washed with 600 mM sucrose and 20 mM Tes-NaOH buffer (pH 7.0) by resuspension and recentrifugation, and finally suspended in 40 ml of the same buffer. The cells were disrupted by passage through a French pressure cell at 40 MPa. After addition of 0.0001% (w/v) DNase I (Sigma Chemical Co., DN-EP) and 1 mM phenylmethylsulfonyl fluoride, the homogenate was made up to a sucrose concentration of 48% (w/v) by adding 0.65 volume of 90% (w/v) sucrose solution in 10 mM NaCl/5 mM EDTA/10 mM Tes-NaOH buffer (pH 7.0). A 16 ml aliquot of the homogenate in the 48% sucrose was placed at the bottom of a centrifuge tube with an internal volume of 35 ml, overlayered with 6 ml each of 45%, 30% and 15% (w/v) sucrose solutions, and centrifuged at $130000 \times g$ for 18 h at 4°C in a swinging bucket rotor (Hitachi RPS 27). All the sucrose solutions were prepared with 10 mM NaCl/5 mM EDTA/10 mM Tes-NaOH buffer (pH 7.0). The cytoplasmic membranes formed a yellow band in the 30% sucrose layer and the thylakoid membranes formed a green band over the interface between the 45 and 48% sucrose layers. The membranes were withdrawn from the gradients, collected by centrifugation at $300000 \times g$ for 1 h after 3-fold dilution with 5 mM Tes-NaOH buffer (pH 7.0), and washed by resuspension and recentrifugatoin with the same buffer.

Water-soluble cytochromes were released from the thylakoid membranes by ultrasonic treatment at 0°C for 90 s in 5 mM Tes-NaOH buffer (pH 7.0) with a sonic oscillator (Ohtake, 5202), which was operated at 20 kHz and 100 W. The water-soluble cytochromes and the membranes were separated by centrifugation at $300000 \times g$ for 2 h.

Cytochromes in the membrane preparations were analyzed by low-temperature spectrophotometry [14]. Samples were reduced with sodium dithionite or hydroquinone and oxidized with potassium ferricyanide in a 1:1 (v/v)-mixture of glycerol and 100 mM Tes-NaOH buffer (pH 7.0). They were then frozen in liquid nitrogen, allowed to stand at room temperature until devitrification occurred, and cooled again to −196°C. The reduced-minus-oxidized difference spectra were measured with a split-beam spectrophotometer (Shimadzu UV-300) equipped with a low-temperature apparatus under the conditions of 1 mm light path, 1 nm half band width, 9.375 $nm \cdot min^{-1}$ scan rate and 1 s time constant. 15 scans were recorded and averaged with a spectral data processing computer (Shimadzu SAPCOM-1A) to improve the signal-to-noise ratio. Fourth derivative spectra were calculated from the averaged spectra [15] with a sampling interval of 0.2 nm and a differentiating interval of 1.2 nm by a built-in program of the computer.

The membrane-bound and water-soluble cytochromes were also analyzed by room-temperature spectrophotometry under the conditions of 1 cm light path, 1 nm half band width, 37.5 $nm \cdot min^{-1}$ scan rate and 0.2 s time constant. 10 scans were averaged and fourth derivative spectra were calculated with a sampling interval of 0.5 nm and a differentiating interval of 3.0 nm. The amounts of the water-soluble cytochromes were determined using a reduced-minus-oxidized difference absorption coefficient of 20 $mM^{-1} \cdot cm^{-1}$ at the $\alpha$-band maxima [16].

LDS-polyacrylamide gel electrophoresis [17] and detection of heme-containing polypeptide bands with TMBZ and $H_2O_2$ [18] were performed according to Guikema and Sherman [19]. The membrane samples were delipidated with 90% aqueous acetone at 0°C for 5 min prior to solubilization by LDS. The solution of water-soluble cyto-

chromes was placed in a dialysis tube and concentrated by dusting with polyethylene glycol. LDS and TMBZ were purchased from Sigma Chemical Co. Molecular mass standards used were ovalbumin (45 kDa), chymotrypsinogen (25 kDa) and cytochrome *c* (12.5 kDa).

For determination of hemes, the membrane preparations were suspended in 0.2% HCl and shaken with three volumes of 2-butanone [20]. Protoheme in the 2-butanone phase and heme *c* in the aqueous phase were determined by an alkaline pyridine hemochrome method [20]. The reduced-minus-oxidized difference absorption coefficients used were: $\Delta\epsilon^{red-ox}_{549.5-535} = 22.0\ mM^{-1} \cdot cm^{-1}$ for heme *c*, and $\Delta\epsilon^{red-ox}_{555-535} = 24.0\ mM^{-1} \cdot cm^{-1}$ for protoheme [21]. The water-soluble cytochromes were directly subjected to the alkaline pyridine hemochrome method, and the concentrations of protoheme and heme *c* were estimated by calculation from a reduced-minus-oxidized difference spectrum of the hemochromes [21].

For determination of prenylquinones, total lipids were extracted from the membranes according to Bligh and Dyer [22], and the prenylquinones were analyzed by TLC and HPLC. In TLC, precoated silica gel plates containing a fluorescent dye (Merck, 60 $F_{254}$) were used. Prenylquinones were located on the thin-layer plates by irradiation with ultraviolet light of 254 nm [23]. They were identified by their absorption spectra and $R_f$ values in TLC [24]. The TLC plate was first developed with benzene [24], which formed two spots having $R_f$ values of 0.54 for phylloquinone and plastoquinone-9, and 0.11 for 5′-monohydroxyphylloquinone [25]. The plate was then cut and the piece containing plastoquinone-9 plus phylloquinone was placed upside-down and developed three times with ehxane/dioxane (200 : 3, v/v) to achieve complete separation. The silica gel of the quinone zones was scraped off the plates, and the quinones were extracted with ethanol. The quinones were determined spectrophotometrically as described by Barr and Crane [26]. Difference absorption coefficients between oxidized and reduced forms of 15 $mM^{-1} \cdot cm^{-1}$ at 255 nm for plastoquinone-9 and 19 $mM^{-1} \cdot cm^{-1}$ at 248 nm for phylloquinone and 5′-monohydroxyphylloquinone were used.

Prenylquinones were also analyzed by HPLC using a column of Lichrosorb SI 60, 5 μm (Merck) according to Lichtenthaler and Prenzel [27]. The column was 250 mm long and 4 mm in diameter. HPLC was performed with a solvent delivery system (Waters, 6000A) at a flow rate of 3 $ml \cdot min^{-1}$ with a pressure of 11.7 MPa. Elution of prenylquinones was monitored at 260 nm with a spectrophotometric detector (Shimadzu SPD-2A). Plastoquinone-9 and 5′-monohydroxyphylloquinone, purified by TLC as described above, and commercially purchased phylloquinone (Tokyo Chemical Industry Co.) were used as chromatographic standards. Phylloquinone and plastoquinone-9 were eluted from the column with 0.3% dioxane in hexane, at retention times of 4.4 and 6.6 min, respectively. 5′-Monohydroxyphylloquinone was eluted by 1.25% dioxane in hexane at a retention time of 17 min. The quinones were quantitatively determined by measuring the areas under the peaks on the chromatograms and comparing them with those of standards.

Proteins and chlorophyll were determined as described previously [13].

## Results

Cytochromes in the thylakoid and cytoplasmic membranes were studied by measuring oxidation-reduction difference spectra at −196°C. A dithionite-reduced minus ferricyanide-oxidized difference spectrum of the thylakoid membranes (Fig. 1a) showed two peaks at 547.5 and 554.9 nm with shoulders at 556.7 and 560.0 nm. Its fourth derivative spectrum (Fig. 1d) had four major peaks at 547.0, 553.5, 557.2 and 560.2 nm, indicating that the difference spectrum was composed of at least four spectral bands having peak maxima at about these wavelengths. In a dithionite-reduced minus hydroquinone-reduced difference spectrum (Fig. 1b), a peak appeared at 556.8 nm with a shoulder at about 560 nm. Its fourth derivative spectrum (Fig. 1e) had three components with peaks at 553.7, 556.8 and 560.3 nm. Since absorption peaks of photosynthetic cytochromes shift toward shorter wavelengths by 2 to 3 nm upon cooling to −196°C [28,29], they are identified as cytochrome *f*, a low-potential form of cytochrome *b*-559 and cytochrome $b_6$, respectively [29,30]. A hydroquinone-reduced minus ferricyanide-oxidized difference

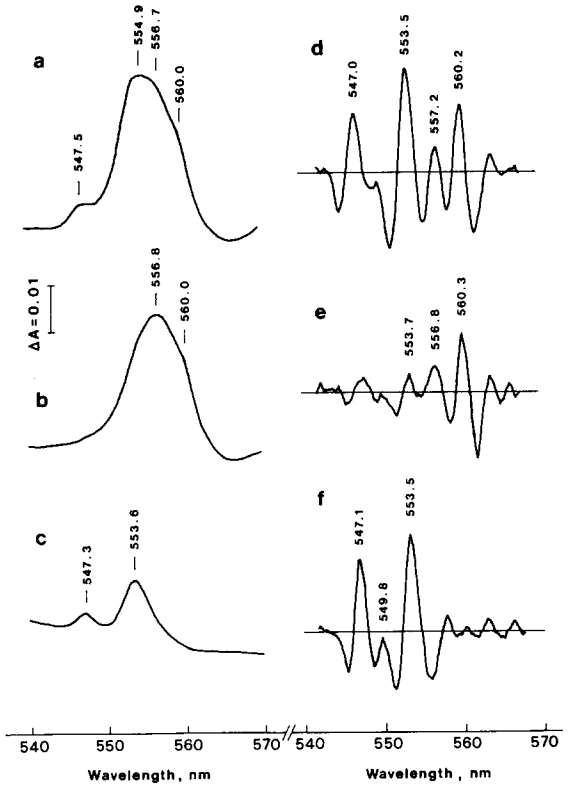


Fig. 1. Reduced-minus-oxidized difference spectra of thylakoid membranes of *A. nidulans* measured at −196°C and their fourth derivative spectra. The concentration of the thylakoid membranes was 0.2 mg chlorophyll *a* per ml, which corresponded to 1.5 mg protein per ml. Final concentrations of hydroquinone and ferricyanide were 5 mM and 2 mM, respectively. In the case of dithionite, a few small grains were added. (a) Dithionite-reduced minus ferricyanide-oxidized difference spectrum; (b) dithionite-reduced minus hydroquinone-reduced difference spectrum; (c) hydroquinone-reduced minus ferricyanide-oxidized difference spectrum; (d), (e) and (f), the fourth derivative spectra of (a), (b) and (c), respectively.

spectrum (Fig. 1c) had two peaks at 547.3 and 553.6 nm, which are characteristic of the split α-band of cytochrome *f* [16,28]. No peak or shoulder was observed at 557 nm, suggesting a lack of the high-potential form of cytochrome *b*-559. Its fourth derivative spectrum (Fig. 1f) had two sharp peaks at 547.1 and 553.5 nm and a minor one at 549.8 nm. The last peak might be due to cytochrome *c*-552 [31] or may originate from an artifact of the fourth derivative method [15]. These results indicate that the thylakoid membranes contain cytochrome *b*-559, cytochrome $b_6$, cytochrome *f*, and possibly a minor proportoin of cytochrome *c*-552. This composition is similar to that of *Nostoc muscorum* [30] and *Phormidium laminosum* [16]. Under our experimental conditions, low-potential cytochrome *c*-549 [31] was not detected spectrophotometrically in the thylakoid membranes. This cytochrome might be trapped in the internal space of the membrane vesicles, and thus not be affected by reductants added to the external medium.

Cytochromes in the cytoplasmic membranes were analyzed in a similar way (Fig. 2). The dithionite-reduced minus ferricyanide-oxidized difference spectrum (Fig. 2a) revealed two peaks at 553.2 and 558.9 nm, and its fourth derivative spectrum (Fig. 2d) had two sharp peaks at 552.9 and 559.4 nm. The dithionite-reduced minus hydroquinone-reduced difference spectrum (Fig. 2b)

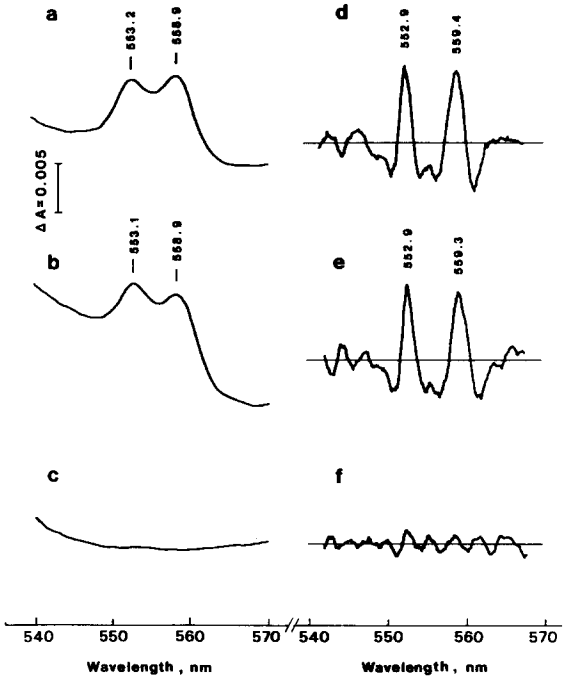


Fig. 2. Reduced-minus-oxidized difference spectra of cytoplasmic membranes of *A. nidulans* measured at −196°C and their fourth derivative spectra. The concentration of the cytoplasmic membranes was 0.7 mg protein per ml. The reductants and the oxidant were added as explained in Fig. 1. (a) Dithionite-reduced minus ferricyanide-oxidized difference spectrum; (b) dithionite-reduced minus hydroquinone-reduced difference spectrum; (c) hydroquinone-reduced minus ferricyanide-oxidized difference spectrum; (d), (e) and (f), the fourth derivative spectra of (a), (b) and (c), respectively.

was the same as the dithionite-reduced minus ferricyanide-oxidized difference spectrum. There was no peak in the hydroquinone-reduced minus ferricyanide-oxidized difference spectrum (Fig. 2c), nor in its fourth derivative (Fig. 2f). The spectral component with a peak at about 553 nm in the cytoplasmic membranes is apparently distinct from cytochrome *f* and water-soluble cytochrome *c*-554 [31], since it cannot be reduced by hydroquinone. The other component with a peak at about 559 nm was not cytochrome $b_6$, since the peak wavelength of the former was invariably shorter by 1 nm than that of the latter. These observations suggest that the cytoplasmic membranes contain two types of cytochromes, or a type of cytochrome having two split α-bands [32]. In either case, these cytochrome(s) are different from any of the cytochromes detected in the thylakoid membranes.

The cytochromes in the cytoplasmic membranes were also studied by room-temperature difference spectrophotometry. Although the dithionite-reduced minus ferricyanide-oxidized difference spectrum showed a single broad peak at 559 nm, its fourth derivative revealed two peaks at about 555 and 561 nm, corresponding to those at 553 and 559 nm at −196°C, respectively (data not shown). There was no peak around 605 and 630 nm in the dithionite-reduced minus ferricyanide-oxidized difference spectrum, suggesting that there was no cytochrome $aa_3$ nor cytochrome *d* in the cytoplasmic membranes.

The water-soluble cytochromes from the thylakoid membranes also were analyzed at room temperature. The dithionite-reduced minus hydroquinone-reduced difference spectrum showed a peak at 549 nm, suggesting that cytochrome *c*-549 was present in the thylakoid membranes. The hydroquinone-reduced minus ferricyanide-oxidized difference spectrum showed a peak at 552 nm, suggesting that cytochrome *c*-552 was present in the thylakoid membranes (data not shown). The molar ratio of cytochrome *c*-549 to *c*-552 was about 13 : 1.

Fig. 3 shows the dithionite-reduced minus untreated difference spectra of alkaline pyridine hemochromes prepared from 2-butanone extracts of the thylakoid and cytoplasmic membranes. In both spectra, there was a peak at 555 nm due to protoheme, but no peak due to heme *a* at 587 nm nor heme *d* at 620 nm. Heme compositions of the thylakoid and cytoplasmic membranes are summarized in Table I. The total heme content of the cytoplasmic membranes was about one-fifth of that of the thylakoid membranes. The cytoplasmic membranes had a protoheme to heme *c* ratio of 5 : 1, suggesting that the major cytochrome(s) in these membranes were of the *b*-type. Table I shows that all the water-soluble cytochromes from the thylakoid membranes were of the *c*-type and that

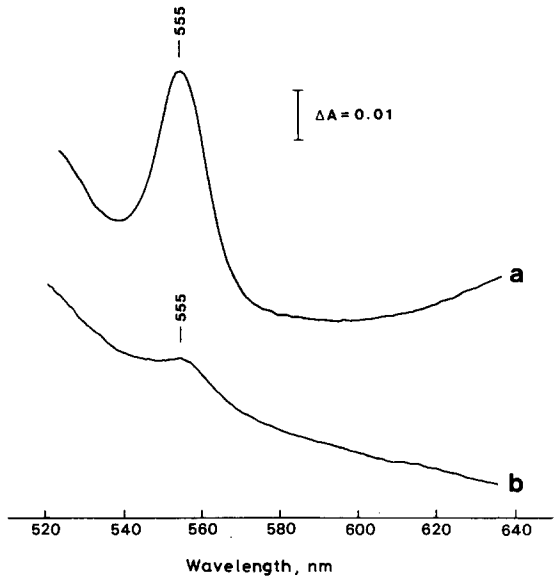


Fig. 3. Dithionite-reduced minus untreated difference absorption spectra of the alkaline pyridine hemochrome prepared from the 2-butanone extract of thylakoid (a) and cytoplasmic (b) membranes which were suspended in 0.2% HCl.

TABLE I

HEME CONTENT OF THYLAKOID AND CYTOPLASMIC MEMBRANES OF *A. NIDULANS*

| Heme | Content (nmol·mg$^{-1}$ protein) | | | |
|---|---|---|---|---|
| | Thylakoid membranes | | | Cytoplasmic membranes |
| | Total | Soluble fraction[a] | Membrane fraction[a] | |
| Protoheme | 1.48 | 0.00 | 1.48 | 0.36 |
| Heme *c* | 0.76 | 0.37 | 0.39 | 0.07 |
| Heme *a* | < 0.01 | 0.00 | < 0.01 | < 0.01 |
| Heme *d* | < 0.01 | 0.00 | < 0.01 | < 0.02 |

[a] The cytochromes were separated into two fractions by sonic oscillation followed by centrifugation.

they can account for about half of the heme *c* in the thylakoid membranes.

Cytochromes in both types of membrane preparations were analyzed also by LDS-polyacrylamide gel electrophoresis followed by TMBZ/$H_2O_2$ staining (Fig. 4). Because of the high lipid content in the cytoplasmic membranes [13], the polypeptide bands were not well separated by gel electrophoresis especially in the low molecular mass region. Therefore, the membrane samples were delipidated prior to electrophoresis. In the thylakoid membranes, five heme-stained bands appeared at molecular mass regions of 34, 29, 19, 13 and 9 kDa (Fig. 4a). The 29 kDa band was converted to the 34 kDa band by heating in a medium containing urea (Fig. 4b). This heme-staining profile is essentially the same as those reported by Guikema and Sherman [19] for the cytochromes

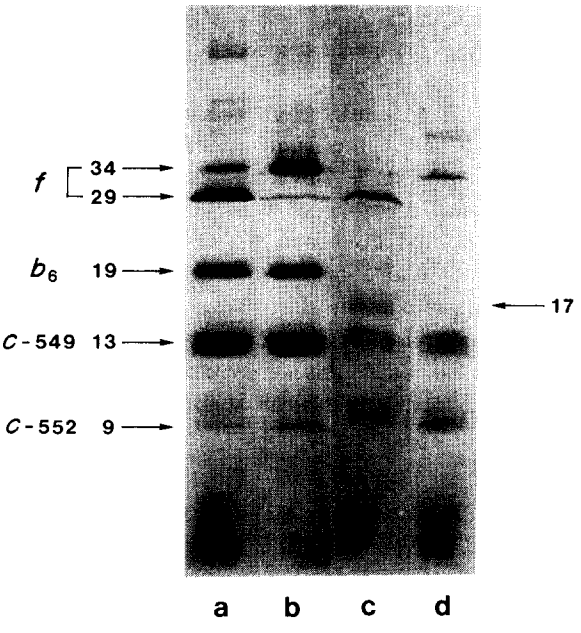


Fig. 4. Analysis of cytochromes in thylakoid and cytoplasmic membranes of *A. nidulans* by LDS-polyacrylamide gel electrophoresis followed by staining with TMBZ/$H_2O_2$. (a) and (b), Thylakoid membranes; (c) and (d), cytoplasmic membranes. The membrane samples of 0.20 mg (a, b) or 0.23 mg (c, d) protein were delipidated with 90% acetone and solubilized in 1% LDS and 8% sucrose at 0°C for 30 min (a, c) or in 1% LDS, 6 M urea and 8% sucrose at 70°C for 5 min (b, d). Samples were subjected to electrophoresis on polyacrylamide gel with a 10–20% gradient. The numbers represent the apparent molecular masses of the stained bands in kilodaltons.

from the total membrane fraction of *A. nidulans* R2 strain. The two bands at 34 and 29 kDa can be ascribed to different conformational states of cytochrome *f*, and the band at 19 kDa to cytochrome $b_6$ [19]. The doublet bands at 13 kDa disappeared from the membranes after ultrasonic treatment and were the main heme-stainable bands in the water-soluble cytochrome fraction from the thylakoid membranes (data not shown). The band at 9 kDa also disappeared after ultrasonic oscillation and was found in the water-soluble fraction (data not shown). These two bands might correspond to cytochromes *c*-549 and *c*-552 which were detected spectrophotometrically as the water-soluble cytochrome components from the thylakoid membranes. The facts that cytochrome *c*-549 (e.g., 14.7 kDa in *Phormidium laminosum* [33]) is larger than cytochrome *c*-552 (about 11 kDa in several species of cyanobacteria [34]) and that cytochrome *c*-549 was found to be the major water-soluble cytochrome component of the thylakoid membranes suggest that the bands at 13 kDa are attributable to cytochrome *c*-549 and the faint band at 9 kDa to cytochrome *c*-552. There was no heme-stained band attributable to cytochrome *b*-559 in the thylakoid membrane preparation.

The staining profiles of LDS-polyacrylamide gel electrophoresis of the cytoplasmic membranes are shown in Figs. 4c and d. When the sample was solubilized at 0°C in the absence of urea, six heme-stained bands appeared at 34, 29, 19, 17, 13 and 9 kDa, five of which were due to the cytochromes found in the thylakoid membranes. Since these cytochromes could not be detected in the reduced-minus-oxidized difference absorption spectrum of the cytoplasmic membranes (Fig. 2), they are assumed to be present in very small amounts. They can be ascribed to contamination of the cytoplasmic membrane preparation by a small proportion of the thylakoid membranes. Another band appeared at 17 kDa, but disappeared when the sample was heated in the presence (Fig. 4d) or absence (data not shown) of urea. The observation that the protoheme to heme *c* ratio was high in the cytoplasmic membranes and the fact that protoheme is easily released from the protein moiety during delipidation [35], denaturation by detergent treatment, and electrophoretic migration [18] suggest that the band at 17 kDa is a

TABLE II

PRENYLQUINONE CONTENT OF THYLAKOID AND CYTOPLASMIC MEMBRANES OF *A. NIDULANS*

Results of two experiments using independent membrane preparations and one experiment using intact cells are presented. The values in parentheses represent the relative contents in molar percentage.

| Prenylquinone | Content (nmol · mg$^{-1}$ protein) | | | | |
|---|---|---|---|---|---|
| | Thylakoid membranes | | Cytoplasmic membranes | | Intact [a] cells |
| | Expt. 1 [a] | Expt. 2 [b] | Expt. 1 [a] | Expt. 2 [b] | |
| Plastoquinone-9 | 4.4 (71) | 4.1 (71) | 5.5 (95) | 7.5 (93) | (68) |
| Phylloquinone | 0.2 (3) | 0.2 (3) | < 0.1 | 0.2 (2) | (5) |
| 5′-Monohydroxyphylloquinone | 1.6 (26) | 1.5 (26) | 0.3 (5) | 0.4 (5) | (27) |

[a] Determined by TLC.
[b] Determined by HPLC.

*b*-type cytochrome and its actual content, relative to those of other cytochromes, should be much higher than predicted from the heme-staining profile. The band at 17 kDa is likely to be due to the cytochrome(s) detected spectrophotometrically in the cytoplasmic membranes.

The prenylquinone contents of the thylakoid membranes and cytoplasmic membranes were compared (Table II). *A. nidulans* contained plastoquinone-9, phylloquinone and 5′-monohydroxyphylloquinone as already demonstrated [24,25,36]. The prenylquinone composition of the thylakoid membrane preparation was very similar to that of the whole cells. Their plastoquinone-9 content, on the protein basis, was comparable to that reported for thylakoid membranes of spinach chloroplasts [37]. The cytoplasmic membranes also contained a substantial amount of plastoquinone-9. Clearly, the prenylquinone composition of the cytoplasmic membranes was different from that of the thylakoid membranes.

## Discussion

Our results show that there are cytochrome(s) specific to the cytoplasmic membranes of *A. nidulans*. This does not agree with the result of Lockau and Pfeffer [9] who claimed that the cytoplasmic membranes from *Anabaena variabilis* do not contain cytochromes. However, Peschek et al. [10], using a cytochemical technique, observed the reduction of tellurite and tetranitroblue tetrazolium chloride by the cytoplasmic membrane in *A. nidulans*. Thus, cytochrome(s) in the cytoplasmic membrane may participate in electron transport reactions. Peschek [38] has also reported that *A. nidulans* contains cytochrome $aa_3$ and that this could explain the cytochrome *c* oxidase activity of its membrane preparation. However, we did not detect heme *a* in either the cytoplasmic or the thylakoid membranes.

The cytoplasmic membranes of *A. nidulans* contain a substantial amount of plastoquinone-9 and are similar, in this respect, to the chloroplast envelope membranes [37]. The prenylquinone may constitute an electron-transport system in collaboration with the cytochrome(s) or may be a biosynthetic pool if the cytoplasmic membrane is, like the chloroplast envelope membrane [39], the site of prenylquinone biosynthesis. A possible physiological function of the prenylquinone in the chloroplast envelope membranes, proposed by Lichtenthaler et al. [37], is as a hydrogen carrier for desaturation of fatty acids and carotenoid precursors. Further study is necessary to elucidate the function of plastoquinone-9 in the cytoplasmic membranes of *A. nidulans*.

The cytoplasmic membranes of *A. nidulans* are similar to the envelope membranes of higher plant chloroplasts in some respects [13]; they are rich in lipids, have low buoyant density, and contain xanthophylls and plastoquinone-9, but little chlorophyll. These observations support the view that the inner envelope membrane of chloroplasts

originates from the cytoplasmic membrane of a cyanobacterium-like symbiont enclosed in primitive eukaryotic cells [40]. One difference between these membranes is that no cytochrome has been detected in the chloroplast envelope membranes [40], while we found cytochrome(s) in the cytoplasmic membranes of *A. nidulans.*

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