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PROPERTIES AND STRUCTURE OF FRACTIONS PREPARED FROM *ANABAENA VARIABILIS* BY THE ACTION OF TRITON X-100*

TERUO OGAWA, LEO P. VERNON AND HILTON H. MOLLENHAUER
C. F. Kettering Research Laboratory, Yellow Springs, Ohio (U.S.A.)
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SUMMARY

Treatment of *Anabaena variabilis* with sonic oscillation produces green membrane fragments which are derived from the photosynthetic system. Further treatment of these fragments with Triton X-100 produces two additional fragments, a blue heavy (H) and an orange light (L) fragment, which are separable by sucrose density gradient centrifugation. Although both the physiological reactions related to photosynthesis ($NADP^+$ photoreduction and O_2 evolution) are missing from these derived particles, physical and biochemical evidence allows an identification of the two fragments. P700 is localized in the H fragment. Fluorescence spectra at room and liquid N_2 temperature, as well as P700 response, show that the H fragment is related to Photosystem 1 and the L fragment is derived from Photosystem 2. On the basis of chlorophyll content the concentrations of carotenoids, lipid and protein are higher in the L fragment, while the ratio of β-carotene to total xanthophylls is higher in the H fragment. Of the total chlorophyll *a*, 80% is found in the H fragment. The electron micrographs of these fragments show that the H fragment consists of flat discs 0.1–0.3 μ in diameter on which many small 100-Å particles are present, while the L fragment consists of small membrane fragments and irregular stranded material 50–60 Å in width.

INTRODUCTION

In contrast to the highly structured chloroplast found in green algae and higher plants, the blue-green algae have a more primitive lamellar structure for their photosynthetic system. The phycobilins found in blue-green algae are easily extracted with aqueous solvents, while green algae and plants contain tightly bound chlorophyll *b* as the accessory pigment. Because of the ease of activating Photosystem 2 by light absorbed by the phycobilins, the blue-green algae have been extensively utilized for study of the two photochemical systems in photosynthesis[1–5], but they have not been examined to see if the photochemical systems can be physically separated through the action of detergents. Such a study would be of value to our general study of the structure and function of the photosynthetic apparatus and we have chosen *Anabaena variabilis* for this investigation.

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulfate.

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The physical separation of two photochemical systems from spinach chloroplasts was first achieved by BOARDMANN AND ANDERSON[6] by the use of digitonin. A number of studies[7–16] have followed, dealing with the properties, composition, and structure of the two fragments. Triton X-100 also produces two such fragments, which have been examined by VERNON and co-workers[17–20] and KE AND VERNON[21]. A small fragment produced by the use of these detergents shows Photosystem-1 activity while the other larger fragment has Photosystem-2 activity. OGAWA, OBATA AND SHIBATA[22] have reported on the separation of two pigment proteins from spinach chloroplasts by the use of polyacrylamide-gel electrophoresis of chloroplasts solubilized with sodium dodecyl sulfate. These pigment proteins have the requisite properties of Photosystems 1 and 2. The same techniques have also been applied to the separation of two pigment proteins from chloroplast (lamellar) fragments obtained from three different species of photosynthetic organisms; *A. variabilis*, *Porphyra yezoensis* and *Phaeodactylum tricornutum*[23].

The present study concerns the chlorophyll-containing fragments obtained from the lamellae of *A. variabilis* by the use of Triton X-100. The original membrane fragments (obtained by sonication of whole cells) are unable to carry out the usual photochemical reactions of photosynthesis although others have reported O_2 evolution and $NADP^+$ photoreduction in similar preparations[24–29]. Treatment of the membrane fragment with Triton X-100, followed by a sucrose density gradient centrifugation, shows the presence of two fragments, which also are inactive in $NADP^+$ photoreduction or O_2 evolution. However, fluorescence spectra and the P700 response show that the heavier fragment is related to Photosystem 1 and the lighter fragment is derived from Photosystem 2. The present paper described the structure and composition of these fragments in comparison with the data obtained for spinach subchloroplast particles.

EXPERIMENTAL

Preparation of samples

Chlorophyll-containing membrane fragments were isolated from Anabaena cells following sonication. Washed cells suspended in 0.01 M Tris–HCl buffer (pH 7.5) were sonicated in a 10 KC Raytheon sonic oscillator for 15 min. The green fraction that sedimented between 10000 and 144000 × *g* contained membrane fragments and starch particles which were washed twice by centrifugation in the same buffer and used for detergent treatment. These fragments, which were free of phycobilins, contained 80 ± 5 % of the total chlorophyll *a* and carotenoids of the cells.

A solution of 1.5 or 0.4 % Triton X-100 in 0.01 M Tris–HCl buffer (pH 7.5) was added to an equal volume of a suspension of green fraction (0.30 ± 0.02 mM in terms of chlorophyll *a* concentration) and the mixture kept for 1 h in an ice bath. The suspension of detergent-treated green fraction was centrifuged for 6 h at 131000 × *g* in the SW-27 rotor of a Spinco Model L 2-65 ultracentrifuge through a discontinuous sucrose density gradient of nine layers (5 to 70%) of sucrose dissolved in 0.01 M Tris–HCl buffer (pH 7.5).

To prepare the membrane fragments for electron micrograph, starch particles were removed from the green fraction by the following procedures. A few crystals of α-amylase (Sigma, Type II-A) was added to a suspension of the green fraction in

0.04 M Tris–HCl buffer (pH 7.0) and the mixture, after incubation for 30 min at 20°, was centrifuged at 144000 × *g* for 30 min. The sediment, which was free of starch particles, consisted almost exclusively of the chlorophyll-containing membrane fragment and was used for electron microscopy.

Fluorimetry and spectrophotometry

Absorption spectra were measured with a Cary-14 recording spectrophotometer. The fluorescence measurements were made with the apparatus described by MAYNE[30]. A tungsten lamp was used for excitation of fluorescence, with blue filters (Corning 5-60 and 4-96) and 5% $CuSO_4$ solution inserted between the lamp and the sample. The fluorescence emitted was analyzed with a Bausch and Lomb grating monochromator and the fluorescence intensity was measured with a solid CO_2-cooled photomultiplier (RCA 7102). To exclude stray light, a red filter (Corning 2–60) was inserted between the monochromator and the sample. All fluorescence spectra shown in this paper are tracings of the original recordings and were not corrected for the efficiency of the monochromator or for the sensitivity of the photomultiplier. Fluorescence spectra were measured at room and liquid N_2 temperatures in a 0.15-mm path length cuvet attached to a cold finger. For low-temperature measurements, the cuvet was cooled with liquid N_2 inside the glass dewar.

The light-induced absorbance changes of the P700 were measured at 430 nm in the flash spectrophotometer described by KE, TREHARNE AND MCKIBBEN[31]. The content of P700 was calculated from the difference spectrum ($Fe(CN)_6^{3-}$ oxidized-*minus*-dithionite reduced samples) measured with a Cary 14 recording spectrophotometer.

Assay of pigments, lipids and proteins

Chlorophyll *a* was determined after extraction of the material with methanol, using an extinction coefficient of $6.58 \cdot 10^4$ mole$^{-1} \cdot$ cm^{-1} at 666 nm (ref. 32). Carotenoids were separated by one dimensional thin-layer chromatography on silica gel, using light petroleum (b.p. 30–60°) containing 30% chloroform as the developer. The following molar extinction coefficients were used for the determination of the carotenoid contents: 1.35, 1.21 and $1.34 \cdot 10^5$ mole$^{-1} \cdot$ cm^{-1} for β-carotene, echinenone and zeaxanthin in light petroleum, respectively[29]. The ε_M value of β-carotene was assumed to be valid for myxoxanthophyll in ethanol.

Lipids were extracted by a chloroform–methanol mixture (3:1, v/v). Extracts were washed by water, evaporated to dryness at 50° and weighed according to the directions of ALLEN *et al.*[33]. Protein was determined by the biuret method[34].

Electron micrographs

Electron micrographs were taken with a Philips EM 200 electron microscope. Negative staining with phosphotungstic acid at pH 5.9 in the presence of 5 mM $MgCl_2$ was used to prepare the samples.

RESULTS

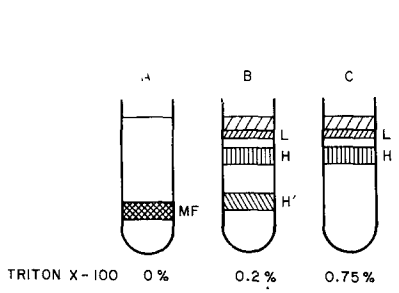
Fragments from photosynthetic apparatus

A. variabilis green fraction (from sonicated cells) treated with 0.75% Triton X-100 produced two fragments which were separated by centrifugation through a discon-

tinuous sucrose gradient, as shown in Fig. 1C. Also shown are tubes containing the green fraction (Fig. 1A), and green fraction treated with 0.2% of Triton X-100 (Fig. 1B). The two fragments produced by the action of 0.75% Triton X-100 are called heavy (H) and light (L) fragments according to their position in the tube. The position of the starch particles in the tube was almost the same as that of the membrane fragment. Accordingly, the H and L fragments were free of starch particles, even without prior amylase treatment, while the membrane fragment prepared by density-gradient centrifugation still contained starch particles. The H fragments were blue while the L fragments appeared orange.

Another orange band was also located near the top of the tube. The fluorescence spectrum of this band showed the presence of solubilized chlorophyll *a* as well as carotenoids. Further treatment of the H fragment with higher concentrations of Triton X-100 did not liberate additional chlorophyll *a*, while more solubilized chlorophyll *a* was produced from the L fragment with high concentrations of Triton X-100. Approximately 80% of total chlorophyll *a* was contained in the H fragment. Treatment of the membrane fragments with 0.2% Triton X-100 produced three fragments (Fig. 1B); two blue bands (the H fragment and another heavier blue band) and the usual L fragment. This heavier blue fragment still contained small amounts of the L fragment, since further extraction of this fragment with Triton X-100 liberated small additional amounts of the L fragment as well as additional H fragment at the expense of the heavier blue fragment. High concentrations of Triton produced more solubilized chlorophyll *a*. Thus, the amounts of solubilized chlorophyll *a* produced by 0.2 and 0.75% Triton X-100 were approx. 30 and 80% of the total chlorophyll *a* in the two orange bands, respectively. To obtain maximum yields of the two fragments, the H fragment was prepared by using 0.75% Triton X-100 while 0.2% Triton X-100 was used for preparation of the L fragment.

The absorption spectra of the H and L fragments are shown in Fig. 2. The absorption maxima of the red band of chlorophyll in the H and L fragments were located at 680 and 672 nm, respectively, and their Soret bands were both located at 440 nm. As seen from Curve B, the spectrum of the L fragment shows a distinct peak at 488 nm and shoulder at 520 nm which is related to carotenoid absorption. On the



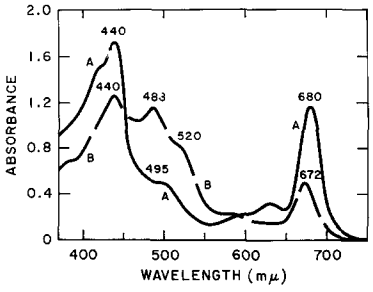


Fig. 1. Distribution of untreated membrane fragment (A) and the fragments produced through the action of 0.2% (B) and 0.75% (C) Triton X-100 after centrifugation for 6 h at 131 000 × *g* through discontinuous gradient of sucrose (5–70%). MF, membrane fragment; H, heavy fragment; L, light fragment.

Fig. 2. Absorption spectra of the H (Curve A) and L (Curve B) fragments. The H and L fragments were prepared using 0.75 and 0.2% Triton X-100, respectively.

other hand, only one shoulder was observed at 495 nm in the spectrum of the H fragment (Curve A). It appears, therefore, that the two fragments differ in their carotenoid composition and, as seen from the difference in the peak position of the red band of chlorophyll *a*, the environment of chlorophyll *a*.

Photochemical activities

The membrane fragment as well as the H and L fragments were inactive in the Hill reaction either with $Fe(CN)_6^{3-}$ or 2,6-dichlorophenolindophenol (DCIP). These fragments were also inactive in $NADP^+$ reduction in the presence of ascorbate and DCIP, which was measured by the method of VERNON, SHAW AND KE[18] using enzymes (ferredoxin, ferredoxin-NADP reductase and plastocyanine) prepared from spinach chloroplasts. Further experimentation using the enzymes prepared from Anabaena is needed.

The P700 response

Fig. 3 shows the light-induced absorbance changes related to the photooxidation of P700 observed for the various fractions in the absence and presence of ascorbate and phenazine methosulfate (PMS). The absorbance changes were followed at 430 nm (this wavelength is free from fluorescence artifacts) and were obtained using 2-sec illumination periods. Without addition of any electron donors, only the membrane fragment showed the light-induced absorbance change of P700. However, the H fragment gave the P700 response in the presence of ascorbate and PMS, which also caused a larger light-induced absorbance change and a faster decay rate for the membrane fragment. An absorbance change at 430 nm was not observed for the L fragment, even in the presence of ascorbate and PMS. These results show that the P700 is localized in the H fragment, and is in an oxidized state (most likely due to the measuring beam used in the spectrophotometer) in the absence of electron donors. The earlier investigations on spinach subchloroplast fragments prepared by the use of digitonin[9,12] or Triton X-100[17–19] showed that the P700 is concentrated in the lighter

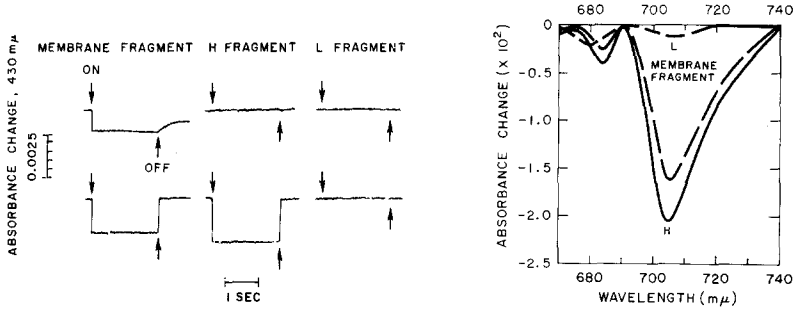


Fig. 3. Light-induced absorbance changes at 430 nm related to P700 in the green membrane fragment and derived fragments. The fragments were suspended in 0.01 M Tris–HCl buffer (pH 7.5). The chlorophyll contents of each sample were 15.2 μg/ml. The samples were examined first without any addition (top tracing) and then with 1 mM ascorbate and 5 μM PMS present (bottom tracing). H fragment, heavy fragment; L fragment, light fragment.

Fig. 4. P700 content in the green membrane fragment and the derived H and L fragments. Difference spectra of $Fe(CN)_6^{3-}$-oxidized *minus* dithionite-reduced samples. Each sample had a chlorophyll content of 27.0 μg chlorophyll *a*/ml and an absorbance of 2.0 at the absorption maximum of the red band. All reagents were added in the solid form in excess.

fragment, which is considered to be one of the important evidences that the light fragment is responsible for photochemical system 1. The results obtained in the present study show that in contrast to spinach chloroplasts it is the H fragment of Anabaena which is responsible for photochemical system 1.

The concentrations of the P700 in each fragment were calculated from the difference spectra between $Fe(CN)_6^{3-}$-oxidized and dithionite-reduced samples. The difference spectra obtained by chemical oxidation and reduction of each sample are shown in Fig. 4, showing that the maximal absorbance changes of P700 occurred at 705 nm and another small peak was observed at 684 nm in the H fragment and membrane fragments. A negligible amount of P700 (which might be due to a contamination of the H fragment) was observed in the L fragment. The ratio of P700 to chlorophyll *a* was calculated to be 1.0 and 0.7 P700 per 100 chlorophyll-*a* molecules for the H and membrane fragment, respectively.

Fluorescence measurements

The fluorescence spectra at room and liquid nitrogen temperature for the green membrane fragment are shown in Fig. 5A. At room temperature, the fluorescence spectrum had two maxima at 685 and 730 nm, with relatively high intensity at 685 nm. Cooling of the green membrane fragment with liquid N_2 resulted in a marked increase of the fluorescence intensity. At —196° the fluorescence spectrum had the major band maximum at 731 nm and only a small peak at 685 nm. A shoulder around 695 nm was barely observable. Curves in Fig. 5B show the fluorescence spectra of the H fragment at room and liquid N_2 temperature, measured with a sample having the same concentration of chlorophyll *a* as the original membrane fragments used for the fluorescence measurement of Fig. 5A. The room temperature fluorescence spectrum of the H fragment had two maxima at 685 and 730 nm. As seen from these room temperature fluorescence spectra of the membrane and H fragments, the fluorescence intensity at 685 nm was much weaker in the H fragment than in the membrane fragment, while differences between the fluorescence intensities at 730 nm were small.

The fluorescence intensity of the H fragment was enhanced at —196°; the spectrum showed a peak located at 731 nm, but lacked the peak at 685 nm and the

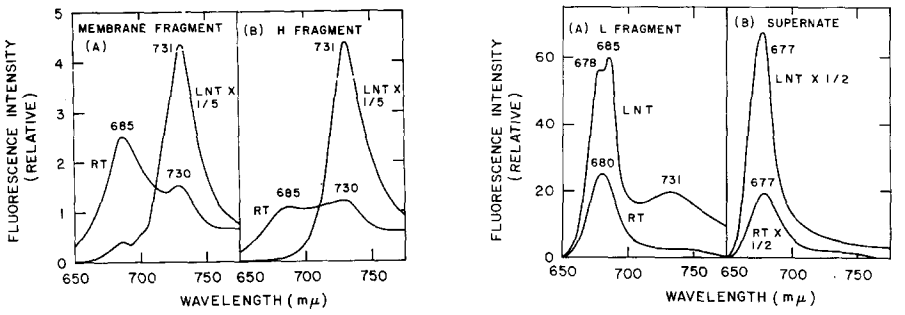


Fig. 5. Fluorescence spectra of the green membrane fragment (A) and the heavy (H) fragment (B) at room (RT) and liquid N_2 temperature (LNT).

Fig. 6. Fluorescence spectra of the light (L) fragment (A) and the orange band at the top of the tube (B) at room (RT) and liquid N_2 temperature (LNT).

shoulder around 695 nm, which was observed in the spectrum of the green membrane fragment taken at liquid N_2 temperature.

Much evidence has been accumulating that the fluorescence bands at 685 and 695 nm observed at −196° are emitted from two forms of chlorophyll *a* in Photosystem 2 of chloroplasts, and the band at 715–735 nm originates from a form of chlorophyll *a* in photochemical system 1 (refs. 35–38). Fluorescence properties have recently been reported for the spinach subchloroplast fragments obtained by the use of digitonin[10,13] and Triton X-100[21], which also support the above concept on the origin of these emission bands. MURATA AND TAKAMIYA[39] have shown that chlorophyll *a* in photochemical system 2 has the major emission band at 685 nm at room temperature.

The data on the fluorescence spectra obtained in this study, therefore, indicate that the H fragment from Anabaena is responsible for Photosystem 1 and does not contain appreciable pigment of Photosystem 2.

Fig. 6 shows the fluorescence spectra of both the L fragment (A) and the orange band found at the top of the tube (B) at room and liquid N_2 temperatures. The room temperature fluorescence spectrum of the L fragment showed a peak at 680 nm. At liquid N_2 temperature, the major band appeared at 685 nm and shoulders appeared at 678 and 731 nm. The orange band from the top of the tube was highly fluorescent and had only one peak at 677 nm, both at room and liquid N_2 temperatures, which shows that this band consists mainly of solubilized chlorophyll *a*. In the fluorescence spectrum of the L fragment, obtained at liquid N_2 temperature, the shoulder at 678 nm might be due to some contamination of solubilized chlorophyll *a*, while the emission band at 685 nm most likely comes from the chlorophyll *a* which has the emission band at 685 and 695 nm in the membrane fragment, though the fluorescence intensity was higher in the L fragment than in the membrane fragment and a shoulder was not observed at 695 nm in the fluorescence spectrum of the L fragment. These data, along with lack of a demonstrable Photosystem 2 in the H fragment, strongly suggest that the L fragment is responsible for Photosystem 2 of the original photosynthetic membrane.

Composition

Carotenoids of the H and L fragments were assayed by thin-layer chromatography of the extracts. Quantitative data obtained from the analyses are summarized in Table I, in which the chlorophyll *a* content is taken at 100 for each fragment. As seen from this table, the ratio of carotenoids to chlorophyll *a* is much higher in the L fragment than in the H fragment. However, the ratio of β-carotene to xanthophylls is higher in the H fragment and the oxygenated carotenoids occur primarily in the L fragment. This uneven distribution of carotenoids in the two fragments resembles the data obtained for Components I and II prepared earlier from Anabaena membrane fragments[23]. The two derived fragments have been analyzed for their lipid and protein content. The results, shown in Table I, show that more protein and lipid were found in the L fragment than in the H fragment.

Structure

The H and L fragments produced through the action of Triton X-100 have been examined in the electron microscope, using the technique of negative staining. Fig. 7A

TABLE I

COMPOSITION OF ANABAENA FRAGMENTS

*Component**	*Heavy fragment*	*Light fragment*
β-Carotene	9	16
Echinenone	6	23
Zeaxanthin	0.3	2
Myxoxanthophyll	0.7	40
Total carotenoids	16	81
β-Carotene/total xanthophylls	1.3	0.25
Lipid**	0.3	4.2
Protein**	0.5	3.7

* Carotenoids in nmoles per 100 nmoles chlorophyll *a*.
** Total lipids or proteins in mg per 100 nmoles chlorophyll *a*.

shows a micrograph of the green fraction that sedimented between 10000 and 144000 × *g* by centrifugation of sonicated cells, showing the presence of flat discs 40–300 nm in diameter (on which appear many small particles of about 100 Å in diameter) and small uniform particles which are 50–100 nm in diameter. Treatment of this fraction with 2.5% Triton X-100 followed by centrifugation at 144000 × *g* for 30 min gave a transparent colorless residue, an electron micrograph of which is shown in Fig. 7B; only the small uniform particles observed in Fig. 7A are present. Addition of KI–I_2 solution (2% KI, 0.2% I_2) to the colorless residue gave a bluish-brown color, showing that these particles are starch. When the starch particles in the green fraction were digested by α-amylase only the green membrane fragment was collected by subsequent centrifugation. Fig. 7C shows a micrograph of such a membrane fragment preparation, showing the presence of the flat discs observed in Fig. 7A. It was not necessary to remove the starch particles prior to Triton X-100 treatment since the starch particles

TABLE II

DESCRIPTION OF ANABAENA FRAGMENTS

Fraction	*Color*	*Nature*	*Properties*
Membrane fragment from sonicated cells	Green	Comminuted membrane	Inactive in Hill reaction. Shows P700 reaction.
H (heavy); after detergent	Blue	Photosystem 1; membrane fragments with 100 Å subunits visible	P700 present. Inactive in $NADP^+$ reduction. Contains 80% of chlorophyll *a*.
L (light); after detergent	Orange	Photosystem 2; irregular stranded material	Lower ratio β-carotene/xanthophylls. Inactive in Hill reaction.
Above L fraction in gradient	Orange	Solubilized pigment (carotenoids, chlorophyll *a*)	—

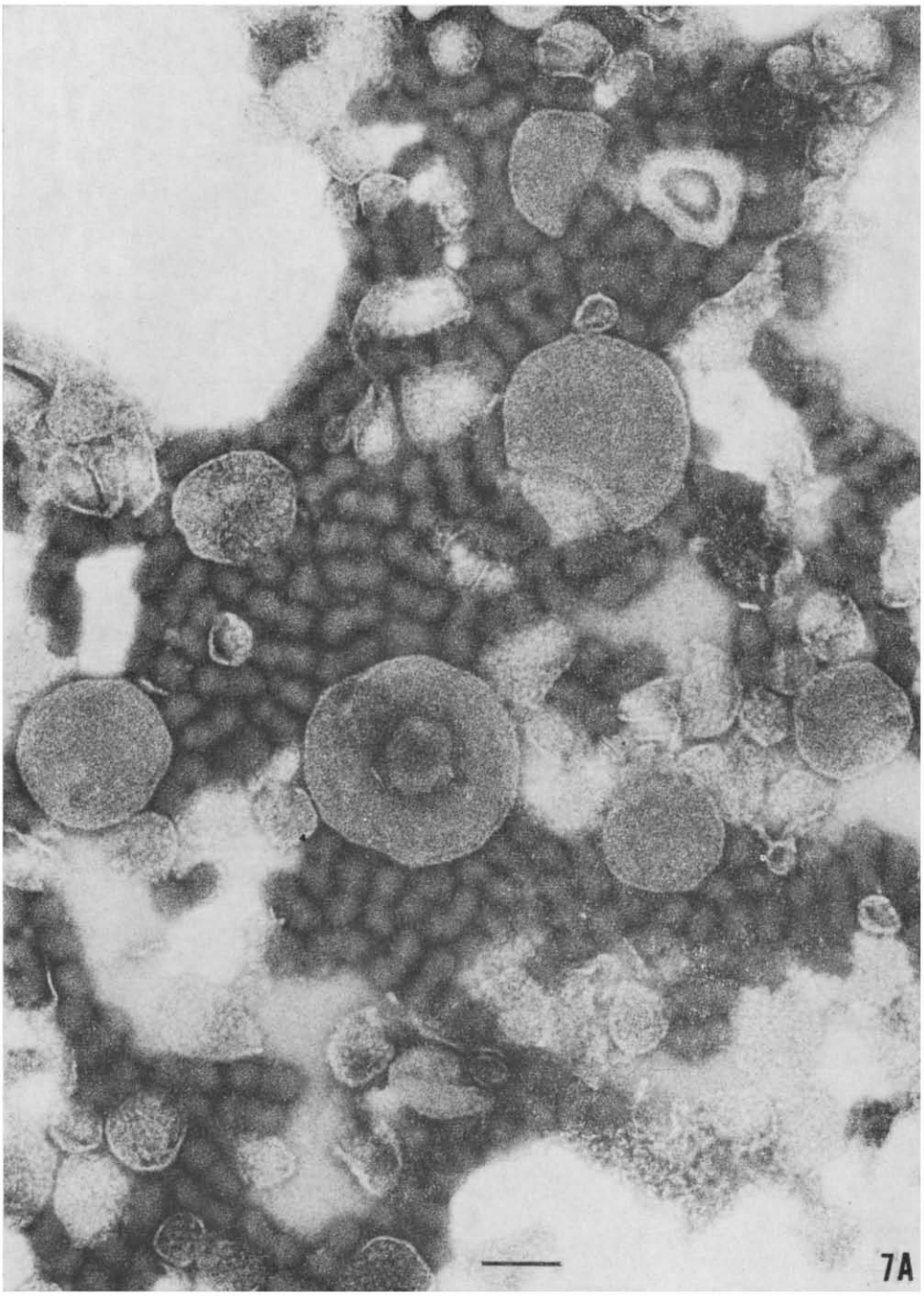
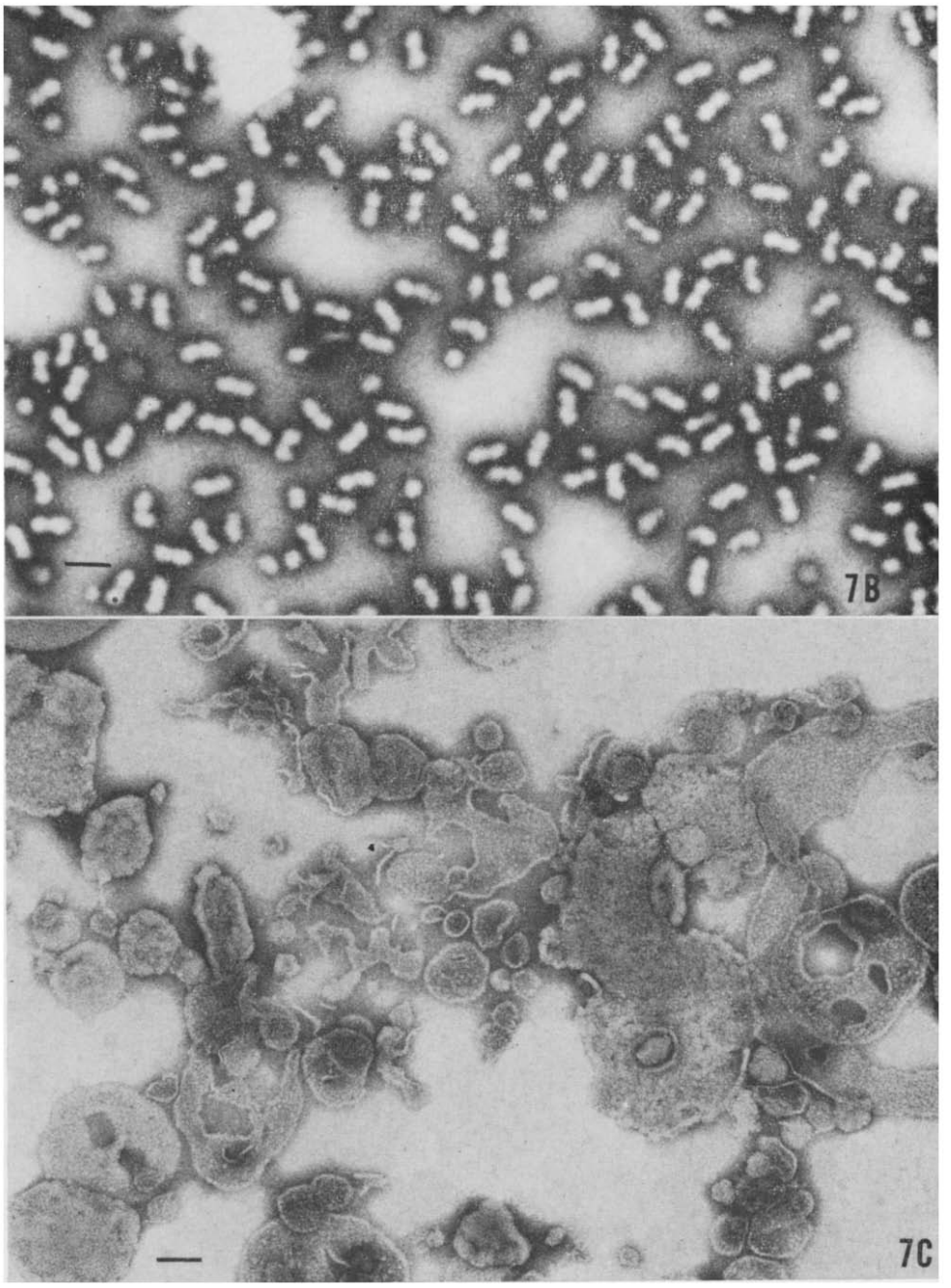


Fig. 7. Electron micrograph of the green fraction that sedimented between 10000 and 144000 × *g* by centrifugation of sonicated cells of *A. variabilis*, showing the presence of both membrane fragments and starch granules (A). Electron micrographs of isolated starch particles (B) and membrane fragments (C) obtained from the green fraction after digestion with amylase to remove the starch



granules. The membrane fragments present in (A) and (C) consist of flat discs 40–300 nm in diameter on which appear many particles of about 100 Å in diameter. Negative staining with phosphotungstic acid at pH 5.9 in the presence of 5 mM $MgCl_2$ was used to prepare the sample. The bar represents 1000 Å.

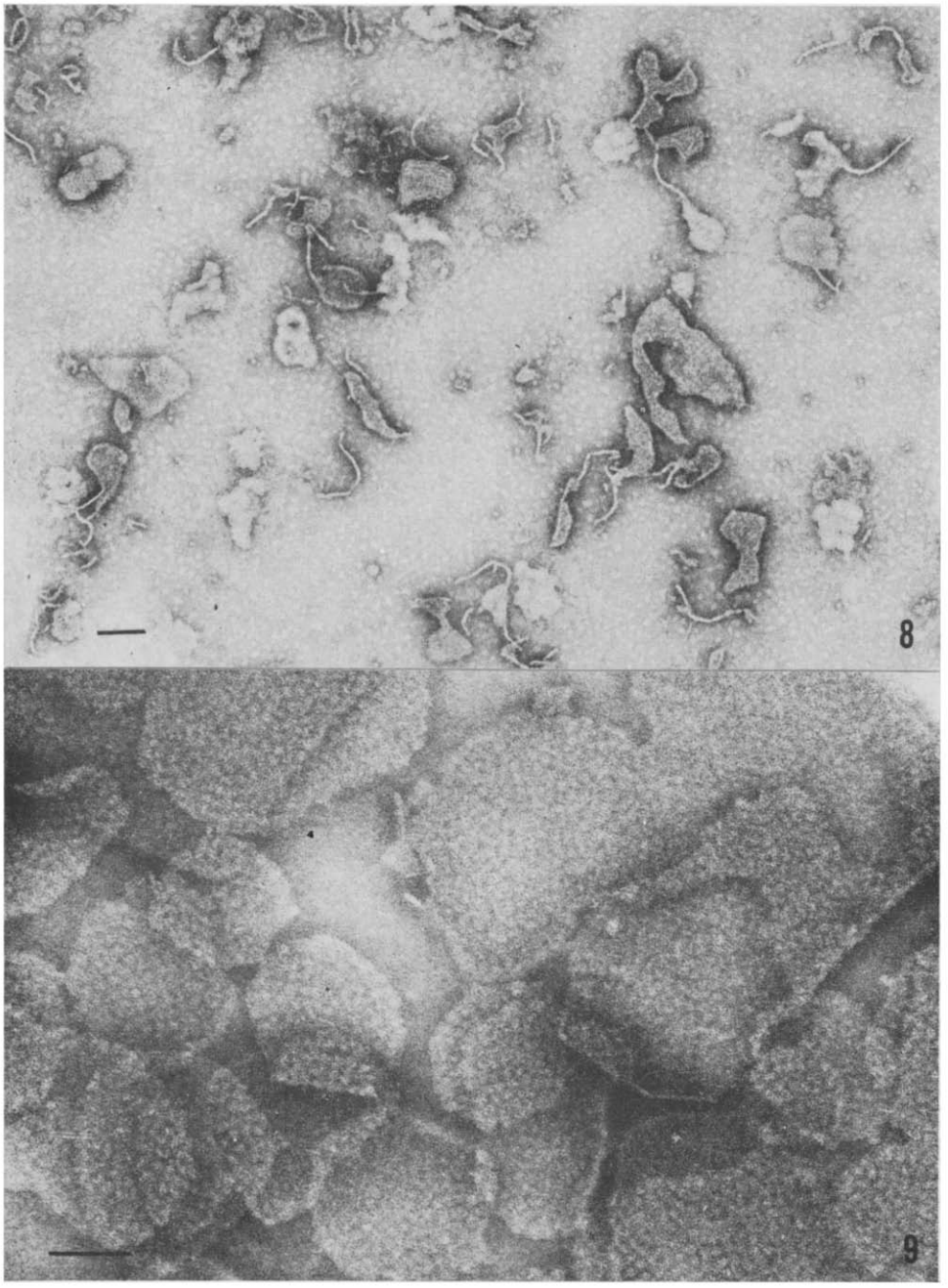


Fig. 8. Electron micrograph of the L fragment prepared by the action of 0.2 % Triton X-100. The electron micrograph was obtained by the technique of negative staining as in Fig. 7. Small strands, 50–60 Å in width, and a few aggregates of these strands or irregular membrane fragment are present. The bar represents 1000 Å.

Fig. 9. Electron micrograph of the H fragment prepared by the action of 0.75 % Triton X-100. The electron micrograph was obtained by the technique of negative staining as in Fig. 7. Flat discs are 0.1–0.3 μ in diameter, on which appear many small particles of about 100 Å in diameter. The bar represents 1000 Å.

were not dissolved by Triton X-100 treatment and were easily removed from the H and L fragments during the sucrose density gradient centrifugation.

The L fragment, as shown in Fig. 8, consists of small strands 50–60 Å in width, as well as some membrane fragments. Also present are a few aggregates of these strands. On the other hand, the H fragment consists of flat discs 0.1–0.3 μ in diameter, on which many small particles of about 100 Å in diameter attach, as shown in Fig. 9. The appearance of these small particles resembles that of the coupling factor isolated from spinach chloroplasts by HOWELL AND MOUDRIANAKIS[40]; however, we could not remove these particles by the procedures outlined in their paper. The nature of these particles is still unknown. The properties of the various fractions obtained from Anabaena are shown in Table II.

By comparing the structures of the two fragments obtained from spinach chloroplasts by Triton X-100 treatment[20] with those shown above for Anabaena, it is apparent that the two photosynthetic membrane systems respond differently to the action of the detergent. With spinach chloroplasts the smaller particle removed from the membrane contains Photosystem-1 activity, while the residual membrane continuum contains the components of Photosystem 2. Just the opposite is observed with Anabaena. The exact reason for this difference is not apparent on the basis of our meager understanding of the architecture of the photosynthetic membrane system. It might be, however, that Photosystem 2 of the blue-green algae is situated more on the exterior of the membrane system, so that it can be in close proximity to the accessory phycobilins, and thus efficiently receive the energy transferred from the accessory pigment. Obviously, further experimentation is needed on this interesting system.

DISCUSSION

The ability of Triton X-100 to fragment the photosynthetic apparatus from chloroplasts of higher plants and bacterial chromatophores has been well documented[20], and the present investigation shows that a similar response is obtained with a blue-green alga. In the present case, two fragments are produced which have most of the characteristics found in similar fragments prepared from spinach chloroplasts. Considering its content of P700 and its fluorescence properties, one fragment contains Photosystem 1, while the fluorescence spectra from the other fragment indicates it is from Photosystem 2.

Since *A. variabilis* contains no chlorophyll *b*, and the phycobilins are washed out during the preparation of the original green membrane fragment, the only pigments encountered in the various fragments are chlorophyll *a* and carotenoids. The usual distribution is encountered, with over 80% of the chlorophyll *a* present in the H fragment containing Photosystem 1, and the majority of the xanthophylls located in the L fragment containing Photosystem 2. The high carotenoid content of the L fragment makes it appear orange in color. Although the L fragment contains more β-carotene than the H fragment on a chlorophyll-*a* basis, when the higher content of chlorophyll *a* in the H fragment is considered, it is apparent that there is more β-carotene in the H fragment on a protein basis.

The appearance of the two fragments in the electron microscope differs from what has been observed with similar fragments from spinach chloroplasts. In the case of *A. variabilis*, the fragment containing Photosystem 1 is the heavier fragment and

is more membranous in nature than is the fragment containing Photosystem 2, which consists of small stranded material and small, irregular pieces of membrane. This indicates that for this alga the Photosystem-2 apparatus is removed from the original membrane by the detergent, leaving the Photosystem-1 apparatus on the membrane matrix. Thus, either photosystem can be removed from the membrane by Triton X-100 depending upon the nature of the original membrane. In the present case the occurrence of phycobilins, which are accessory pigments for Photosystem 2 and are located as phycobilisomes on the exterior portion of the membrane, may be related to the ease of removal of Photosystem 2 by the detergent.

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