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EVIDENCE FOR TWO TYPES OF *P*-700 IN MEMBRANE FRAGMENTS FROM A BLUE-GREEN ALGA*

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SUMMARY

The mathematical analysis described in the preceding paper (Biochim. Biophys. Acta (1977) 460, 65–75), in which the steady-state photooxidation of *P*-700 was compared with overall electron flux in Photosystem I chloroplast fragments, was applied to membrane fragments from the blue-green alga *Nostoc muscorum* (Strain 7119) noted for their high activity of both Photosystem I and Photosystem II. The same analysis, which gave good agreement between the photooxidation of *P*-700 and the overall light-induced electron flux (measured as $NADP^+$ reduction) in Photosystem I chloroplast fragments, revealed in the algal membrane fragments two *P*-700 components: one responding to high light intensity (*P*-700 HI), the photooxidation of which was in good agreement with the overall electron flux (measured as $NADP^+$ reduction by reduced 2,6-dichlorophenolindophenol), and the other component responding to low light intensity (*P*-700 LI), the photooxidation of which was not correlated with the reduction of $NADP^+$ by reduced 2,6-dichlorophenolindophenol.

INTRODUCTION

In the preceding paper [1] we described the theoretical basis for a spectrophotometric method that relates the steady-state redox levels of a component electron carrier in a photosynthetic electron-transport system to the overall electron flux through the system. The validity of this method was demonstrated by the correspondence between the effects of light intensity on the photooxidation of *P*-700 and the overall electron flux as measured by the rate of reduction of $NADP^+$ in chloroplast fragments enriched in Photosystem I and lacking Photosystem II activity, but supplied with an artificial electron donor.

The method was used here to investigate the relation between the photooxidation of *P*-700 and the overall light-induced electron flux, measured by the rate of

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; TCIP, 2,3,6-trichlorophenolindophenol; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; PDA, *p*-phenylenediamine; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

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$NADP^+$ reduction in membrane fragments (Fraction C) from the blue-green alga *Nostoc muscorum*, Strain 7119 [2]. Unlike the chloroplast fragments used in the preceding study, the algal membrane fragments were noted for their high activity of both Photosystems I and II [2].

The results indicate that the algal membrane fragments contain two types of *P*-700, only one of which is correlated with the photoreduction of $NADP^+$ by reduced DCIP.

METHODS

Membrane fragments (Fraction C) were prepared from the blue-green alga *N. muscorum*, Strain 7119, as previously described [2]. The procedures for the preparation of spinach ferredoxin and ferredoxin-$NADP^+$ reductase and measurements of chlorophyll concentration and rate of light-induced $NADP^+$ reduction by dichlorophenolindophenol (DCIP) in the presence of excess ascorbate were as described in the preceding paper [1]. For measurements of *P*-700, an Aminco DW-2 spectrophotometer was used in the dual-wavelength mode with the measuring wavelength set at 703 nm and the reference wavelength at 730 nm. (Note exception in Fig. 5.)

RESULTS

Effect of light intensity on oxidation of P-700

The preceding paper [1] gave the theoretical derivation and experimental validation of Eqn. 1, which expresses a relationship between the steady-state photooxidation of the reaction-center pigment, *P* (i.e. the formation of P_{ox}) and the incident light intensity, *I*.

$$P_{ox} = \frac{P_t}{\frac{k \cdot P_t}{\Phi_r \cdot I} + 1} \tag{1}$$

P_t is the sum of the oxidized and reduced fractions of *P* ($P_t = P_{ox} + P_{red}$); *k* is the rate constant for the dark reduction of *P* as a function of the concentration of the electron donor; and Φ_r is the relative quantum efficiency [1]. *P*, with its subscripts, is equated here with *P*-700 and its corresponding oxidized and reduced fractions; the electron donor was the reduced form of DCIP.

The steady-state, light-induced oxidation of *P*-700 was attained in a few seconds. When the absorbance change of *P*-700 at the steady-state was plotted against the log of light intensity, the result was not a series of sigmoidal curves, as expected from theory and as verified experimentally with Photosystem I chloroplast fragments [1]. Instead, the obtained curves were composites of two distinct sigmoidal sections, a result that pointed to the presence of two *P*-700 components (Fig. 1). It is to be noted that the absorbance change due to the photooxidation of *P*-700, labelled $\Delta A_{703\ nm} \times 10^3$ in Fig. 1 and elsewhere, stands for $-(A_{703\ nm} - A_{730\ nm}) \cdot 10^3$.

Fig. 1 shows that one *P*-700 component was associated with lower light intensities and that a second *P*-700 component was associated with higher light intensities. In the preceding paper [1], a parameter, $I_{\frac{1}{2}}$, was introduced and defined as the light

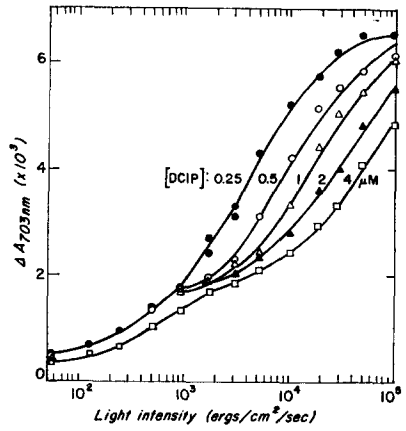


Fig. 1. Effect of light intensity on steady-state absorbance changes at 703 nm. The reaction mixture contained *Nostoc* membrane fragments (Fraction C) equivalent to 15 μg/ml of chlorophyll *a*, 50 mM Tricine buffer (pH 7.7), 2.5 mM $MgCl_2$, 20 mM sodium ascorbate, 5 mM NADP, 1 μM DCMU, 20 μM spinach ferredoxin, a saturating amount of spinach ferredoxin-$NADP^+$ oxidoreductase, and DCIP as indicated. Absorbance changes were measured at room temperature as described under Methods. The actinic light was provided by a tungsten-iodine lamp with two blue-green filters (Corning 4-96) and appropriate neutral density filter combinations as described in the preceding paper [1].

intensity that induces the photooxidation of one-half of P_t (*P*-700). The $I_{\frac{1}{2}}$ values for the first *P*-700 component were lower than those for the second *P*-700 component and were unaffected by concentrations of DCIP in the range examined, from 0.25 to 2 μM. The second component had higher $I_{\frac{1}{2}}$ values and these were dependent on DCIP concentration.

The presence of two *P*-700 components in the algal membrane fragments was also suggested by the double reciprocal plot of $1/P_{ox}$ (shown here as $1/\Delta A_{703\,nm}$)

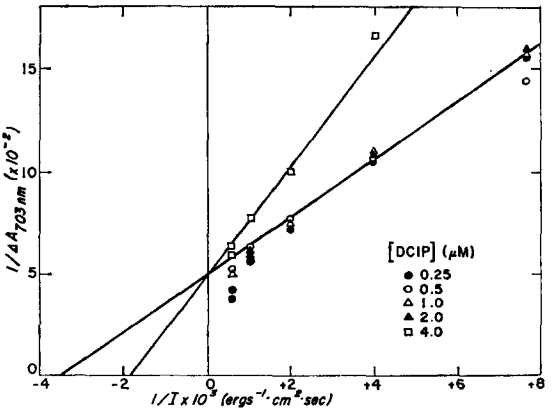


Fig. 2. Double reciprocal plot of the low light intensity portion of the data from Fig. 1. See text for details.

versus $1/I$ (Fig. 2). The plot was based on Eqn. 2, which was derived and tested in the preceding paper [1].

$$\frac{1}{P_{ox}} = \frac{k}{\Phi_r} \cdot \frac{1}{I} + \frac{1}{P_t} \tag{2}$$

In the low range of light intensity between 10^2 and 10^3 ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} (that would correspond to $1/I$ values between $+10$ and $+1$ on the X-axis of Fig. 2), the double reciprocal plot gave a straight line, as would be expected for a single P-700 component. However, at higher light intensities (i.e. at $1/I$ values smaller than $+1$ on the X-axis of Fig. 2), the points diverged from a straight line, as would be expected if two P-700 components were involved.

The conclusion that $\Delta A_{703\text{ nm}}$ measured at the higher light intensities was the sum of absorption changes of two P-700 components, one photooxidized predominantly at high incident light intensities (P-700 HI) and one photooxidized even at low light intensities (P-700 LI), was tested by separating and comparing the two components. P-700 LI was determined from the plot in Fig. 2, in which the straight lines obtained at low light intensities gave a Y-axis intercept equal to $5 \cdot 10^2$. According to Eqn. 2 and the discussion in the preceding paper [1], this intercept corresponds to $1/P_t$, i.e. to a value of $2 \cdot 10^{-3}$ for P_t, which in turn is equal to the absorbance change due to P-700 LI. This absorbance change due to P-700 LI, when subtracted from the total absorbance change at 703 nm, gave a modified absorbance change ($\Delta A'_{703\text{ nm}}$) which was due to only the P-700 component (P-700 HI), associated with high light intensity.

At different concentrations of DCIP, a double reciprocal plot of $1/\Delta A'_{703\text{ nm}}$ against $1/I$ gave, in accordance with Eqn. 2, a series of straight lines with a common y-axis intercept (Fig. 3). The plot showed that the photooxidation of the high light intensity P-700 component was affected by the concentration of DCIP.

The response of the high light intensity P-700 component (P-700 HI) and the relative lack of response of the low light intensity P-700 component (P-700 LI) to DCIP concentration is shown in a different manner in Fig. 4. The $I_{\frac{1}{2}}$ values used in

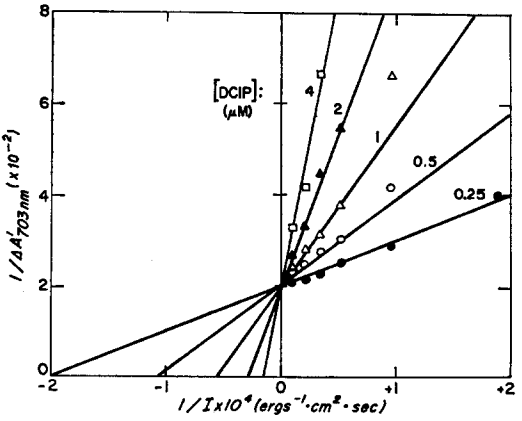
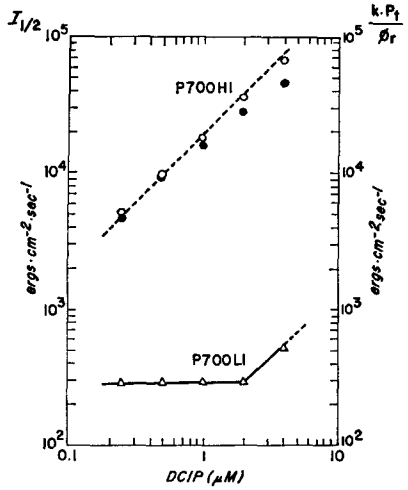


Fig. 3. Double reciprocal plot of the high light intensity portion of the data from Fig. 1. For modification of absorbance changes ($\Delta A'$) and other details, see text.



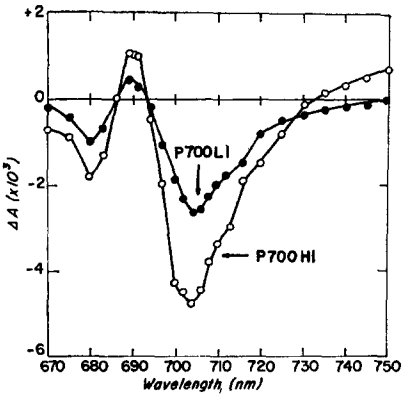


Fig. 4. Comparison of $I_{\frac{1}{2}}$ and $k \cdot P_t/\Phi_r$. See text for explanation.

Fig. 5. Difference spectra of *P*-700 HI and *P*-700 LI. Absorbance changes (light minus dark) were measured in an Aminco DW-2 spectrophotometer used in the split-beam mode. Experimental conditions were as in Fig. 1 except that 0.5 μM DCIP was used throughout. For *P*-700 LI (full circles) absorbance changes were measured when the actinic light intensity was $1 \cdot 10^3$ ergs · cm^{-2} · s^{-1}. These absorbance changes were subtracted from those measured at a high light intensity ($5 \cdot 10^4$ ergs · cm^2 · s^{-1}) to give the absorbance changes (empty circles) due solely to the *P*-700 HI component.

Fig. 4 were determined as reciprocals of the *x*-axis intercept (cf. ref. 1) obtained from Fig. 3 for *P*-700 HI and from Fig. 2 for *P*-700 LI. The $I_{\frac{1}{2}}$ values for *P*-700 HI (empty circles) were proportional to DCIP concentration, whereas the $I_{\frac{1}{2}}$ values for *P*-700 LI (triangles) were unaffected by DCIP concentrations lower than 4 μM (Fig. 4). (The full circles in Fig. 4 will be discussed later.)

A comparison of the absorption spectra of the two photooxidized *P*-700 components is shown in Fig. 5. The comparison was made by measuring the absorption spectra of the algal membrane fragments in the region of 670 to 750 nm, once at a representative high light intensity and once at a representative low light intensity. The absorption spectra at the low and high light intensities each had a major peak at 703 nm, a minor peak at 680 nm, and a trough at 690 nm (Fig. 5) and were so closely similar and typical of *P*-700 as to leave little doubt that both were indeed absorption spectra of components of *P*-700.

Correlation between photooxidation of P-700 and NADP$^+$ reduction

Our next objective was to compare the electron flux measured by the photooxidation of each of the two *P*-700 components with the total light-induced electron flux as measured by the reduction of NADP$^+$. The comparison was made by the same method that was found valid for the Photosystem I chloroplast fragments dealt with in the preceding paper [1], i.e. the values of $I_{\frac{1}{2}}$ obtained from measurements of *P*-700 photooxidation were compared with the values of $I_{\frac{1}{2}}$ obtained from Eqn. 3,

$$I_{\frac{1}{2}} = \frac{k \cdot P_t}{\Phi_r} \qquad (3)$$

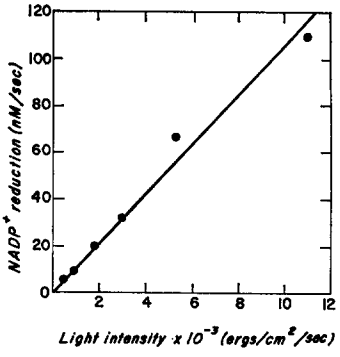


Fig. 6. Effect of light intensity on rate of $NADP^+$ reduction. Experimental conditions as in Fig. 1 except that 4 μM DCIP was used.

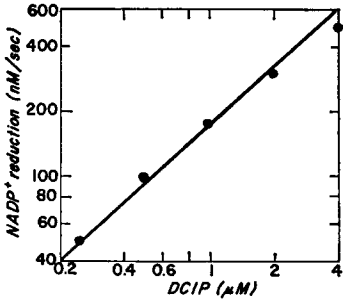


Fig. 7. Effect of DCIP concentration on rate of $NADP^+$ reduction. Actinic light intensity was $5 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. Other experimental conditions as in Fig. 1.

where rates of $NADP^+$ reduction were used to determine $k \cdot P_t$ and Φ_r, terms that have the same meaning as in Eqn. 1.

Φ_r, the relative quantum efficiency, was determined as the slope of the straight line plot of the rate of $NADP^+$ reduction against light intensity, in the presence of high concentrations of DCIP (Fig. 6). The values of $k \cdot P_t$ were determined from the plot of the rate of $NADP^+$ reduction against DCIP concentration, at a high intensity of incident light (Fig. 7). Finally, the $k \cdot P_t$ values for each concentration of DCIP were divided by Φ_r, to give the respective $I_{\frac{1}{2}}$ values, according to Eqn. 3. The $I_{\frac{1}{2}}$ values so obtained when plotted against DCIP concentration (full circles in Fig. 4) were in good agreement with the $I_{\frac{1}{2}}$ values obtained from measurements of photooxidation of *P*-700 HI (empty circles) but not *P*-700 LI (triangles in Fig. 4).

Effect of other electron donors

In all of the experiments discussed so far, reduced DCIP was the only electron donor. Of the other electron donors tested with the algal membrane fragments, TCIP and DAD were as effective as DCIP, whereas TMPD and PDA were poor electron donors for $NADP^+$ reduction. Spinach plastocyanin and mammalian cytochrome *c* were also ineffective as electron donors. In testing their effectiveness, each of these substances was, like DCIP, added in catalytic amounts and was kept in the reduced state by an excess of ascorbate.

The effect of the different electron donors on the $I_{\frac{1}{2}}$ values obtained from measurements of *P*-700 photooxidation is illustrated by the double reciprocal plots of $1/\Delta A_{703\ nm}$ versus $1/I$ in Fig. 8 (compare with Fig. 2). The electron donors fell into two groups: group 1, which included the poor electron donors, TMPD, PDA, and ascorbate alone, gave straight line plots with no divergent points, indicating that these electron donors reacted poorly but equally with both *P*-700 components; group 2, which included the effective electron donors DCIP, DAD, and TCIP, gave a straight line plot that was similar to that for DCIP alone (shown in Fig. 2), i.e. a straight line without divergence of points at low light intensities but with considerable scatter of

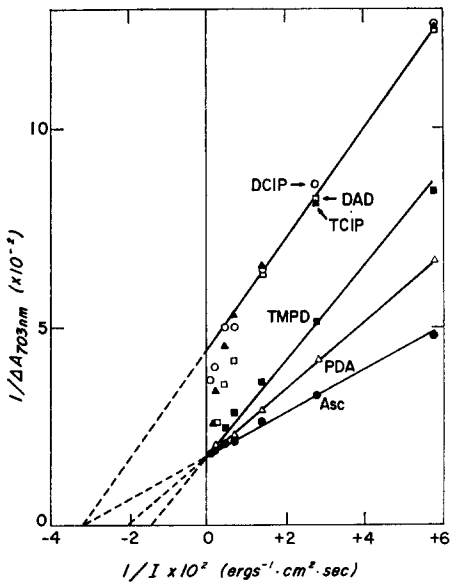


Fig. 8. Double reciprocal plot of the effect of light intensity on *P*-700 absorbance changes using different electron donors. Experimental conditions were as in Fig. 1 except that 10 mM ascorbate was used throughout. Where indicated, 0.25 μM DCIP, 0.25 μM TCIP, 10 μM DAD, 10 μM TMPD, or 10 μM PDA was used.

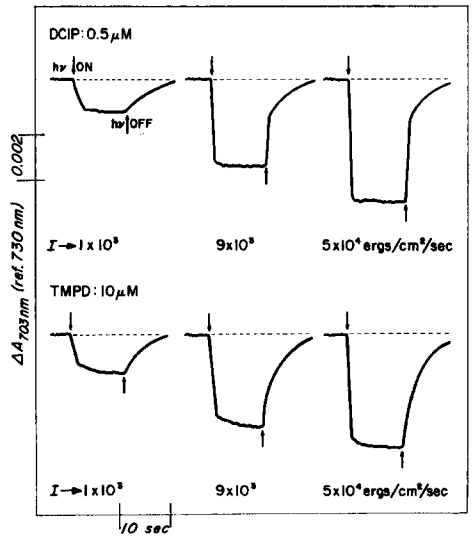


Fig. 9. Effect of light intensity on recovery kinetics of *P*-700. The traces are examples of data used for plotting Fig. 8.

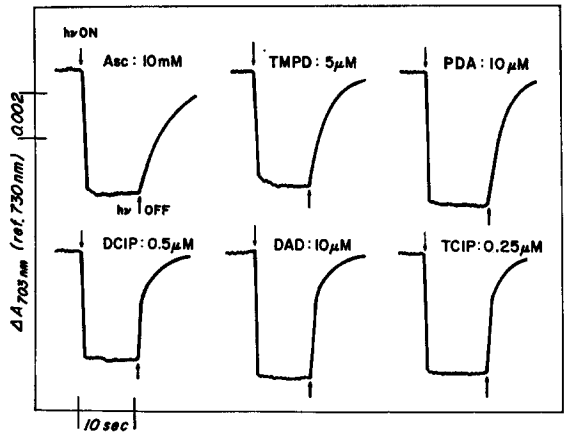


Fig. 10. Effect of different electron donors on the recovery kinetics of *P*-700. The traces are examples of the data used for plotting Fig. 8. Light intensity, $5 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}.

points at high light intensities. The electron donors in this group seemed to react effectively with *P*-700 HI but poorly with *P*-700 LI.

Recovery kinetics of P-700

Similar differences between the two groups of electron donors were also apparent from measurements of recovery kinetics of *P*-700 after the actinic light was turned off, that is, from kinetic measurements of *P*-700 reduction in the dark, following its prior oxidation in the light. With reduced DCIP as the electron donor, and after prior low intensity illumination, only a slow phase of *P*-700 reduction was observed (Fig. 9, upper left). After a higher intensity illumination, a biphasic reduction pattern of *P*-700 was apparent, consisting of a rapid phase whose amplitude increased with an increase in intensity of prior illumination and a slow phase whose amplitude was not affected by the intensity of prior illumination (Fig. 9, upper middle and right). It seems likely that the slow phase corresponds to *P*-700 LI and the fast phase corresponds to *P*-700 HI.

With TMPD as the electron donor, *P*-700 reduction kinetics appeared monophasic at all three intensities of prior illumination (Fig. 9, lower traces). These results are consistent with the earlier interpretation that TMPD reduces both *P*-700 components at the same slow rate.

Fig. 10 summarizes the *P*-700 recovery kinetics of the two groups of electron donors used in the determination of $I_{\frac{1}{2}}$ values (Fig. 8). Group 1 (ascorbate, TMPD, and PDA) gave a uniformly monophasic *P*-700 recovery pattern (Fig. 10, upper traces). By contrast, group 2 (DCIP, DAD, and TCIP) gave biphasic *P*-700 recovery patterns.

DISCUSSION

The previous paper [1] described a mathematical analysis that relates the actinic light intensity to the steady-state redox level of a reaction-center pigment (*P*-700) and to the overall light-induced electron flux as measured by $NADP^+$ reduction.

When applied to Photosystem I chloroplast fragments, the analysis gave a good agreement between the photooxidation of total *P*-700 and the overall electron flux as measured by the rate of $NADP^+$ reduction [1]. In the present investigation, however, the same analysis, when applied to algal membrane fragments possessing both Photosystem I and Photosystem II activities, revealed the existence of two *P*-700 components, one responding to a high intensity of actinic light (*P*-700 HI) and one responding to a low intensity of actinic light (*P*-700 LI). A correlation was obtained between the overall light-induced electron flux from reduced DCIP to $NADP^+$ and the photooxidation of *P*-700 HI but not *P*-700 LI. We conclude, therefore, that *P*-700 HI, but not *P*-700 LI, was the reaction-center pigment involved in the light-induced reduction of $NADP^+$ by reduced DCIP.

The photoreduction of $NADP^+$ by reduced DCIP is widely regarded as involving a segment of the electron-transport chain that is responsible for the photoreduction of $NADP^+$ by water [3]. According to this view, evidence for the involvement of a *P*-700 component in the photoreduction of $NADP^+$ by DCIP would also constitute evidence for the involvement of the *P*-700 component in the photoreduction of $NADP^+$ by water. However, other work with the same algal membrane fragments renders this conclusion less certain; thus, redox changes in cytochromes were associated with photoreduction of $NADP^+$ by water but not with the photoreduction of $NADP^+$ by reduced DCIP [4]. Furthermore, high concentrations of $MgCl_2$ strongly inhibited the photoreduction of $NADP^+$ with DCIP without significantly affecting the photoreduction of $NADP^+$ with water [5].

It is possible, therefore, that, when electron flow from water is blocked, reduced DCIP (or another artificial electron donor to Photosystem I) activates an artificial electron-transport pathway that results in the reduction of $NADP^+$ via some carriers that do not normally participate in the photoreduction of $NADP^+$ with water. Other evidence will therefore be sought before a conclusion is reached about the role of *P*-700 HI in the photoreduction of $NADP^+$ with water. Evidence will also be sought for a role of *P*-700 LI in the photosynthetic electron transport of algal membrane fragments.

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