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Microalgal Plasma Membranes Purified by Aqueous Two-Phase Partitioning

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ABSTRACT

Detailed analysis of plasma membrane chemistry calls for isolation and purification of plasma membranes from cell homogenates. There are few published reports describing isolation of plasma membranes from microalgae. We have developed techniques and modified established procedures to successfully obtain sufficient quantities of membranes from three species of marine microalgae. The aqueous two-phase partitioning technique was successfully used to purify the plasma membranes. The collection of highly purified plasma membranes requires optimization of procedures for each species under investigation. Plasma membranes partition to the upper phase in optimal two-phase systems, as indicated by vanadate-sensitive K^+-Mg^{2+} ATPase activity. Other marker enzyme activities were insignificant or undetectable indicating little or no contamination by other membrane fractions. Detailed procedures for membrane isolation as well as composition of optimal two-phase systems for all three species are reported.

Key Words: aqueous two-phase separation, microalgae, plasma membrane, cell fractionation

INTRODUCTION

In all cells, the cytoplasmic (plasma) membrane separates living portions of the cell from the surrounding environment, detects and responds to changes in the environment, and controls passage of materials in and out of the cell. In eukaryotic cells, intracellular membranes separate regions into functional compartments, as well as anchoring multi-enzyme complexes, ribosomes, and other macromolecular structures such as cytoskeletal

elements and structural proteins. Rather than homogeneous, static structures, membranes are dynamic, responsive arrays that participate in metabolic events of living cells. Membrane structure and function are not uniform from one region to the next, but instead show marked differences in molecular composition and associated enzymatic activity, reflecting biochemical specialization of different cell compartments. Extensive studies on cells of higher plants and animals have revealed specific patterns of enzyme localization associated with different membrane fractions.

The plasma membrane of a unicellular microorganism possesses all the necessary components allowing it to sense and respond to signals in its surrounding environment. Therefore, the plasma membrane of a unicellular microorganism is the site of a myriad of metabolic events distinct from events and processes occurring in other membrane fractions of the cell. Detailed understanding of early events as cells respond to environmental stress requires focus on the plasma membrane, as opposed to analysis of bulk membranes from throughout the cell.

Structural and functional differences between various membrane-bound compartments within a cell can be studied through separation of their associated membranes and subsequent investigation of purified fractions. These investigations include, but are not limited to: analysis of protein composition, measuring activity of membrane-bound enzymes, measurements of lipid bilayer fluidity, measurements of ion transport across membranes, or detailed analyses of membrane lipid composition. Consequently, isolation of purified membrane fractions has found broad application in many diverse fields of investigation.

Widell and Larsson (1981) introduced partitioning in aqueous Dextran-polyethylene glycol (PEG) two-phase systems as a method for plasma membrane purification from plants. Since their introduction, aqueous two-phase systems have found wide use in separation of biological materials (Albertsson 1986). Partitioning of bio-materials in aqueous two-phase systems is a simple, yet sensitive method for the purification of cell constituents including proteins, nucleic acids, membranes, and cell organelles (Walter and Johansson 1994). This technique has been applied to bacteria (Schütte et al. 1994), fungi (Kopperschläger 1994), algae (Flynn et al. 1987), higher plants (Larsson et al. 1987), and animals (Joelsson and Tjerneld 1994).

Before utilizing the aqueous two-phase partitioning method to obtain plasma membrane preparations it must be optimized for the particular species to be used. Since much of the literature describes the use of this technique on higher plants, we also wished to determine whether this technique was broadly applicable to photosynthetic microorganisms. The aqueous two-phase partitioning method is presented below, describing preparation of plasma membrane fractions from three species of marine microalgae: *Tetraselmis suecica* (Prasinophyceae), *Chaetoceros gracilis* (Bacillariophyceae), and *Isochrysis* aff. *galbana* (Prymnesiophyceae).

MATERIALS AND METHODS

Microalgal Culture

T. suecica (UTEX 2286), *C. gracilis* (UTEX 2375), and *I.* aff. *galbana* (UTEX 2307) were obtained from the University of Texas culture collection. All organisms were grown axenically in modified ASP-2 media (Provasoli et al. 1957). All media contained the following vitamins: 1 $\mu g \bullet L^{-1}$ biotin, 200 $\mu g \bullet L^{-1}$ thiamine-HCl, and 1 $\mu g \bullet L^{-1}$ vitamin B_{12}. Media for *C. gracilis* also contained 30 $mg \bullet L^{-1}$ sodium silicate, 26.3 $\mu g \bullet L^{-1}$ sodium selenate, and 3.1 $\mu g \bullet L^{-1}$ lithium chloride. The cultures were grown under constant illumination at 25°C using our state-of-the-art computer-controlled photobioreactor (turbidostat) systems (Hearn 1994). Our bioreactor system continuously monitors turbidity of the growing cell suspension. When turbidity exceeds a predetermined set point, the system activates a peristaltic pump to add fresh medium thereby diluting the cell suspension back to the pre-set turbidity. Thus turbidity of the suspension remains constant (within ±1%) while volume increases. Rate of addition of fresh medium is a measure of growth rate. This culture method provides for analyses a large standing crop of microalgal suspension in balanced exponential growth for extended periods of time (Hearn 1994).

Because this system maintains very stable growth conditions, the physiological and biochemical states of the culture remain constant. As a result, growth rates of our test species are maintained constant as well. Intrinsic growth rates of 0.05 to 0.06 $doublings \bullet hour^{-1}$ for each of the test species can be continued for many months. The system allows us to easily reproduce identical culture conditions from experiment to experiment.

Preparation of Membranes

All chemicals were obtained from Sigma-Aldrich. Cells from 250 ml culture (50-80 mg dry weight) were collected by centrifuging at 7000g for 5 min. Cells were washed by resuspending in 25 ml cell washing buffer (0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5) and centrifuging again at 7000g for 5 min. The cells were processed immediately or stored frozen at -80°C for later use. All of the following steps were performed on ice or at 2°C. Cells were resuspended in 25 ml freshly made homogenization buffer (0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5, 5 mM Na_2EDTA, 5 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonylfluoride (PMSF), and 2.5% (w/v) polyvinylpyrrolidone (PVP). Cells were homogenized by ultrasonication to achieve at least 85% cell breakage. The homogenate was then centrifuged at 14,000g for 30 min. This yielded a pellet consisting of whole cells, whole organelles, and cell walls, and a supernatant containing plasma membranes and other intracellular membranes. The pellet was discarded while the supernatant was centrifuged at 100,000g for 30 min. This yielded a crude microsomal pellet, which was resuspended by hand homogenization in 3 ml homogenization buffer. This suspended fraction contained 4-5 mg protein determined by the method of Bradford (1976). Bovine serum albumin was used as the standard.

Purification of Plasma Membranes

The resuspended microsomal pellet from 250 ml of culture suspension was applied to a two-polymer phase system giving a 10 g final weight (Table 1). In the first set of experiments phase mixtures containing 5.9-6.7% (w/w) Dextran T500 and 5.9-6.7%

(w/w) PEG 3350 mixtures were used. Stock solutions containing 22.8% (w/w) Dextran T500 in 0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5 and 22.8% (w/w) PEG 3350 in 0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5 were added to the phase mixture to achieve the desired polymer concentration. In the second set of experiments, Dextran T500 and PEG 3350 mixtures were held constant (concentrations as determined from the previous set of experiments) while NaCl concentration in the phase mixture was varied between 0-30 mM. A stock solution of 0.79 M NaCl in 0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5 was added to the phase mixture to give the desired salt concentration. Each system was mixed by 25-30 inversions of the tube and centrifuged at 1,000g for 5 min. to facilitate phase setting. The top phase was carefully removed with a Pasteur pipette without disturbing the interface. The material at the interface was removed with the lower phase. Both phases were diluted ~5-fold with membrane wash buffer (0.3 M sucrose, 50 mM Tris-HCl, pH 7.5) and membranes were collected at 100,000g for 60 min. Pelleted membranes from both phases were resuspended in 1 ml storage buffer (0.3 M sucrose, 5 mM Tris-HCl, pH 7.0). Isolated membranes were either analyzed immediately or stored frozen at -80°C for later use.

Enzyme Assays

Cyanide insensitive, NAD(P)H cytochrome-*c* reductase and cytochrome-*c* oxidase were assayed spectrophotometrically at 25°C by following the reduction or oxidation of cytochrome-*c* at 550 nm as described by Briskin et al. (1987). In higher plants such as oats and corn, these enzyme assays are markers for endoplasmic reticulum and mitochondria, respectively (Hodges and Leonard, 1974; Gallagher and Leonard 1982). Potassium-stimulated, magnesium-dependent adenosine triphosphatase (K^+-Mg^{2+} ATPase) was assayed by the method of Briskin et al. (1987). This assay marks the plasma membrane (Hodges and Leonard, 1974). The reaction mixture contained 3 mM Tris-ATP, 4 mM $MgSO_4$, 50 mM KCl, 50 mM Mes-Tris, pH 6.5, and 25 μg protein in a final volume of 1 ml. Latent inosine diphosphatase (IDPase) activity was assayed according to the method of Briskin et al. (1987). This assay marks Golgi membranes (Ray et al. 1969). The reaction mixture consisted of 3 mM sodium-inosine diphosphate (Na-IDP), 4 mM $MgSO_4$, 50 mM KCl, 50 mM Tris-Mes, pH 7.5, and 25 μg protein in a final volume of 1 ml. The activity is determined on freshly isolated membranes and after 6 days of storage at 2-4°C. Latent IDPase represents the difference between initial activity and activity observed upon cold storage. Both the K^+-Mg^{2+} ATPase and IDPase assays contained 0.1 mM sodium molybdate and 1 mM sodium azide to inhibit mitochondrial and other non-specific phosphatases (Briskin et al. 1987). The reactions were carried out at 25°C for 1 hr and then stopped by the addition of Ames stopping and color reagent. The released P_i was measured by the method of Ames (1966). The Ames stopping and color reagent contained 7.3 % (w/v) SDS (phosphate free).

RESULTS

Plasma Membrane Purification

Ultrasonication was used to break the algal cells and differential centrifugation was used to separate whole organelles, unbroken cells, and cell wall material from the remainder of the cell membranes. Cell membranes were then pelleted and partitioned by an aqueous Dextran-PEG two-phase system (Figure 1). Optimization of the two-phase partitioning

technique was achieved by first varying the concentrations of Dextran and PEG in the absence of NaCl. Once optimal concentrations of Dextran and PEG were found they were kept constant while NaCl concentration was varied. Following partitioning of membranes between the two phases, membranes of each phase were collected by centrifugation. Purity of isolated membrane fractions were then judged by criteria described below. Two membrane fractions were subsequently used for analysis: the upper phase and lower phase fractions taken from the aqueous two-phase system.

Purity of the Isolated Upper Phase Fraction

Specific enzyme activities characteristic for specific organelle membranes were measured in each fraction. The upper phase (plasma membrane) fraction from the optimal two-phase system from each of the three species was enriched in K^+-Mg^{2+} ATPase activity (Table 2). This type of ATPase activity is considered a specific marker for the plasma membrane, as discussed below.

Total enzyme activities characteristic for specific organelle membranes were also calculated for upper and lower phase fractions obtained by optimal phase partition of the microsomal fraction . These total activities were compared to total activities of marker enzymes obtained from the microsomal fraction (see Table 3). For each species a relatively high percentage of K^+-Mg^{2+} ATPase activity can be seen in the upper phase. High relative activities of the other marker enzymes are seen in the lower phase. This data indicates adequately purified plasma membrane fractions can be obtained from the three algal species.

For each species as the concentrations of Dextran and PEG were increased, a greater percentage of ATPase activity could be found in the upper phase (Figures 2, 3, and 4). Concentrations of Dextran and PEG were chosen that resulted in a high percentage of K^+-Mg^{2+} ATPase activity and comparably lower percentages of latent IDPase, cytochrome *c* oxidase, and NAD(P)H cytochrome *c* reductase activities in the upper phase. Concentrations of 6.4%, 6.5%, and 6.4% Dextran and PEG produced the best results for *T. suecica*, *C. gracilis*, and *I.* aff. *galbana*, respectively.

Enhanced purification of plasma membranes was accomplished by addition of NaCl to the two-phase system (Figures 5, 6, and 7). Optimal NaCl concentrations chosen for the three species were 8 mM, 24 mM, and 8 mM for *T. suecica*, *C. gracilis*, and *I.* aff. *galbana*, respectively. For each species cytochrome *c* oxidase activity was undetectable in the upper phase fraction at optimal NaCl concentration. This demonstrates insignificant mitochondrial contamination. 20% or less of the latent IDPase activity was present in the upper phase at optimal NaCl concentration for each species demonstrating very little contamination by Golgi membranes. At optimal NaCl concentrations a greater percentage of NAD(P)H cytochrome *c* reductase activity was found in the upper phase when compared with other contaminating marker enzymes, indicating possible ER contamination of the plasma membrane preparations. According to Briskin et al. (1987), greater specificity of this marker for the ER can be achieved by determining the component of activity that is insensitive to 1 μM antimycin A. However, we found little change in activity of NAD(P)H cytochrome *c* reductase in the upper phase fractions taken from our optimal two-phase systems upon the addition of antimycin A (data not shown).

DISCUSSION

Traditionally, linear and discontinuous density gradients, after disruption of cells, have been used for purification of the plasma membrane (Hodges et al. 1972). However, these methods are not adapted universally for green tissue because of heavy contamination of plasma membrane fractions by fragmented chloroplasts and other organelles. The aqueous two-phase partitioning method is superior to traditional methods because it separates membrane vesicles according to surface properties and not according to size or density (Larsson et al. 1987). Concentrations of Dextran, PEG, and NaCl are of critical importance to the success of plasma membrane purification. Anions partition preferentially into the lower phase (mainly Dextran), causing the lower phase to become negatively charged, and the upper phase to be positively charged (Larsson 1983). This difference in charge is believed responsible for selective partitioning of the plasma membrane into the upper phase. Optimal composition of the two-phase system yields plasma membrane preparations from angiosperms with purities estimated at about 95% (Larsson 1985; Sandelius et al. 1986) compared to the 50-75% purity achieved with sucrose gradient centrifugation. This procedure is very well suited for use with tissues where contamination by chloroplast membranes is a problem. Plasma membranes distribute almost exclusively to the upper phase even at polymer concentrations where intracellular membrane proteins are found mainly in the lower phase.

In comparison with higher plant literature there are few published reports describing isolation of plasma membrane fractions from microalgae. Our initial attempts proved inadequate due to very low yield of plasma membrane protein. Various homogenization techniques were attempted such as partial cell wall digestion followed by osmotic shock, French Press disruption, and freezing in liquid nitrogen followed by grinding in a mortar and pestle. None of these techniques were suitable as they resulted in either very low percentage of cell breakage or so much homogenization that subsequent fractionation of cell constituents proved impossible. Ultrasonication was eventually chosen because it resulted in a high percentage of the cells being broken in a relatively short period of time. 250 ml samples from all three test species can easily be homogenized in less than 30 minutes using this technique. It then became necessary to develop techniques and modify already existing procedures to optimize the recovery of the crude cell membranes before purifying the plasma membranes. We succeeded in collecting the remaining crude cell membranes by centrifugation only under very high g-force (see Flynn et al. 1986). At this point the composition of our two phase system had to be optimized for each test species to achieve upper phase fractions highly enriched in plasma membranes. Optimization was achieved by performing marker enzyme assays on upper and lower phase fractions taken from a series of two-phase systems differing in the concentration of Dextran, PEG, and NaCl.

The purity of plasma membrane preparations has traditionally been assessed using a combination of marker enzyme assays and stains specific for various cellular membranes. It was first shown by Roland et al. (1972) using electron microscopy that periodic acid-chromic acid-phosphotungstic acid (PCAP) preferentially stains the plasma membrane in whole plant cells. This diagnostic characteristic was then used by Roland et al. (1972) to identify plasma membranes in crude fractions including a number of contaminating intracellular membranes. Subsequently, this PCAP staining fraction was found to be

highly correlated with membrane fractions enriched in K^+-Mg^{2+} ATPase activity. Localization of K^+-Mg^{2+} ATPase activity to the plasma membrane has been demonstrated in this way not only for higher plants (*Zea mays* (Leonard and VanDerWoude 1976), *Avena sativa* (Briskin et al. 1987), and *Dactylis glomerata* (Yoshida et al. 1983)), but also for algae (*Phaeodactylum tricornutum* (Flynn et al. 1987), *Dunaliella salina* (Weiss et al. 1989), and *Nitzschia alba* (Sullivan and Volcani 1974)). Results from these studies are very consistent: plasma membrane fractions are enriched with K^+-Mg^{2+} ATPase activity, this activity is inhibited by vanadate, and a high percentage of the vesicles found in these fractions stain with PCAP. We therefore concluded that membrane fractions enriched in vanadate-sensitive K^+-Mg^{2+} ATPase activity purified from our test species could be definitively identified as plasma membrane fractions.

We noticed that under optimal or near optimal two-phase system conditions, the NAD(P)H cytochrome-*c* reductase activity closely followed K^+-Mg^{2+} ATPase activity (Figures 5, 6, and 7). Cytochrome *c* reductase activity has long been considered a characteristic marker of endoplasmic reticulum (ER) membranes (Hodges and Mills 1986; Peeler et al. 1989). However, recent investigations (Larsson et al. 1994) have noted cytochrome *c* reductase activity associated with higher plant plasma membranes. Larsson et al. (1994) caution this enzyme may not be a reliable marker for ER in highly purified plasma membrane fractions. It is a useful marker for ER in crude fractions when assayed in the absence of detergent, however, since the plasma membrane-bound activity is located at the cytoplasmic surface and therefore mainly latent in crude fractions(Larsson et al. 1994). We suggest (suspect) that the NAD(P)H cytochrome-*c* reductase activity recorded in the pure plasma membrane fractions is not the result of contaminating ER membranes, but rather an enzymatic activity of the algal plasma membranes.

SUMMARY

We have now demonstrated that fractions highly enriched in plasma membranes can be successfully obtained from *T. suecica*, *C. gracilis*, and *I.* aff. *galbana* using the aqueous two-phase partition technique. Plasma membrane fractions from these species are enriched in K^+-Mg^{2+} ATPase activity while other contaminating enzymatic activities are either undetectable or at very low levels, demonstrating these fractions are of high purity. The localized activities of these various marker enzymes in our work are consistent with previous studies (cited above) providing convincing evidence that we can isolate purified plasma membrane fractions. The most suitable partition systems for the three species described here are: 6.4% (w/w) of Dextran T500, 6.4% (w/w) of PEG 3350, and 8 mM NaCl for *T. suecica*; 6.5% (w/w) of Dextran T500, 6.5% (w/w) of PEG 3350, and 24 mM NaCl for *C. gracilis*; 6.4% (w/w) of Dextran T500, 6.4% (w/w) of PEG 3350, and 8 mM NaCl for *I.* aff. *galbana*.

Purities of the plasma membrane fractions discussed in this paper were based on one separation of the crude microsomes using the aqueous two-phase partitioning technique. Enrichment of the upper phase fraction can be increased still more by washing it with fresh lower phase. Fresh lower phase can be obtained from aqueous two-phase systems in which the weight of suspended crude microsomes is replaced by an equal weight of homogenization buffer. Once optimal composition of the aqueous two-phase system is known fresh upper and lower phases can be made in bulk. Virtually chlorophyll free

upper phases can be obtained from both *T. suecica* and *C. gracilis* with only a single phase partition whereas it was necessary to wash the upper phase from *I.* aff. *galbana* with fresh lower phase two additional times.

This procedure is easily amenable to larger quantities of starting material. For example, if 150mg dry weight of material is to be processed, the final weight of the aqueous two-phase system is increased to 20g. This flexibility is very important as uses for isolated fractions will differ according to the field of investigation. Also, cells from different organisms can differ greatly in size and composition. These differences and others will directly affect the amount of starting material that is required to obtain the proper amount of plasma membranes.

The techniques and data reported in this paper lay the foundation for future studies in our laboratory. These studies require detailed characterization of fatty acid composition of isolated plasma membranes taken from our test species. Until now, this type of study has proven difficult as more traditional techniques cannot not yield plasma membrane fractions of high enough purity. Using this technique, we hope to come to a better understanding of the plasma membrane's role in the cell's response to environmental stress.

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Table 1. Stock solutions and procedures for preparing two-phase separation system.

	Desired final concentrations (%, w/w of Dextran and PEG			
Stock Solutions	6.0	6.2	6.4	6.8
1. 22.8% Dextran[a]	2.63g	2.72g	2.81g	2.89g
2. 22.8% PEG	2.63g	2.72g	2.81g	2.89g
3. 4.64g NaCl/100ml	Add 0.1ml (8 mM NaCl)[b]			
4. Membrane fraction	Add 3ml and weigh[c]			
5. Homogenization buffer	Add enough to bring to 10g			

[a] Dextran and PEG stock solutions are both dissolved in 0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5.

[b] For other concentrations of NaCl see fig. 3, 5, and 7.

[c] Microsomes are suspended in homogenization buffer.

Figure 1. Flow chart depicting the steps involved in the purification of plasma membranes using an aqueous two-phase separation. See text for details.

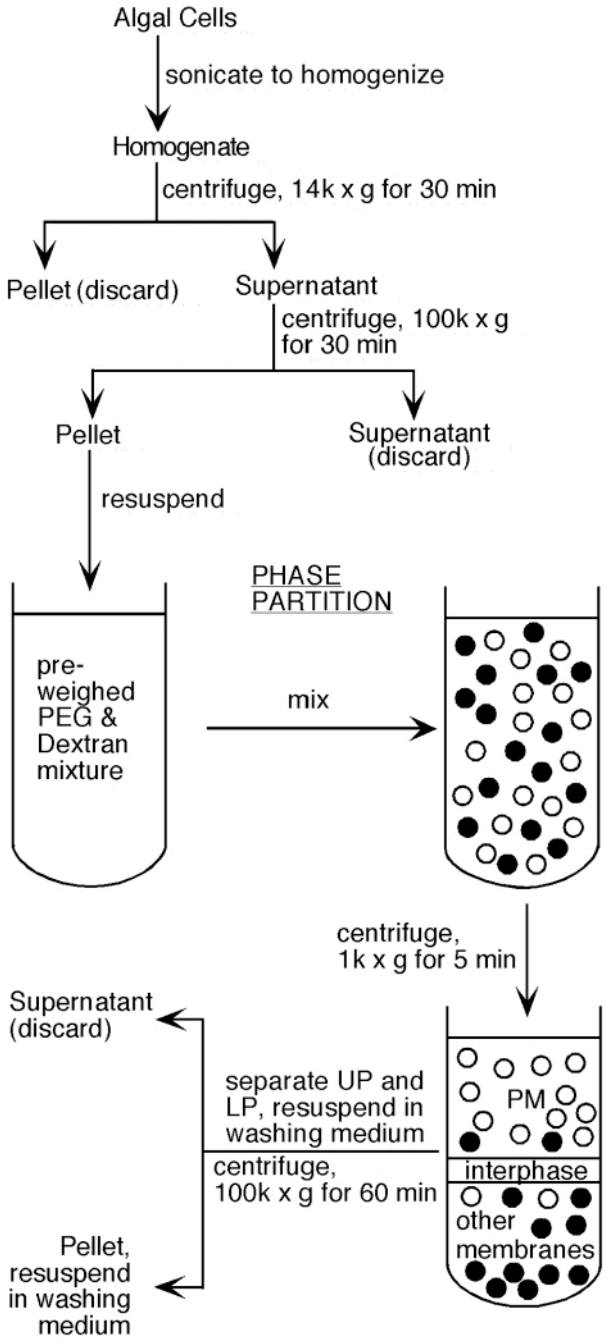

Table 2. Specific activities of marker enzymes in the plasma membrane and the intracellular membranes obtained by optimal phase partitioning of microsomal fractions from microalgae.

Species	Fraction	K^+-Mg^{2+} ATPase (μmol PO^{-4} •mg^{-1}•h^{-1})	Latent IDPase (μmol PO^{-4} •mg^{-1}•h^{-1})	NADPH Cyt *c* reductase (μmol Cyt *c* •mg^{-1}•min^{-1})	Cytochrome *c* oxidase (μmol Cyt *c* •mg^{-1}•min^{-1})
Tetraselmis suecica	Upper Phase	3.05	0.49	5.15	0[a]
	Lower Phase	0.1	2.37	1.30	0.52
Chaetoceros gracilis	Upper Phase	1.12	0	5.51	0
	Lower Phase	0.01	0.02	0.55	0.27
Isochrysis galbana	Upper Phase	11.46	1.02	1.38	0
	Lower Phase	0.24	2.46	0.81	5.13

[a]Undetectable.

Table 3. Comparison of the total marker enzyme activities from the upper phase, lower phase, and microsomal membrane fractions.

Species	Fraction	K^+-Mg^{2+} ATPase (nmol min^{-1})	Latent IDPase (nmol min^{-1})	NADPH Cyt *c* reductase (μmol min^{-1})	Cytochrome *c* oxidase (μmol min^{-1})
Tetraselmis suecica	Upper Phase[b]	51	19	43	0[a]
	Lower Phase	58	81	54	22
	Microsomal	115	105	120	30
Chaetoceros gracilis	Upper Phase	15	0	76	0
	Lower Phase	3	6	22	11
	Microsomal	21	8	111	14
Isochrysis galbana	Upper Phase	158	31	21	0
	Lower Phase	31	116	108	681
	Microsomal	210	153	137	700

[a] Undetectable.

[b] Upper and lower phase fractions were obtained by optimal phase partition of the microsomal fraction.

Figure 2. Effects of increasing polymer concentrations on the partition of different membranes in a microsomal fraction from *Tetraselmis suecica*. Markers used were as follows: K^+-stimulated, Mg^{2+}-dependent Adenosine triphosphatase for plasma membranes (■), Latent – Inosine diphosphatase for Golgi membranes (●), NADPH Cytochrome-*c* reductase for endoplasmic reticulum (▲), and Cytochrome-*c* oxidase for mitochondria (◆). Apart from the polymers, the phase systems contained 0.5 M sorbitol and 50 mM Tris-HCl, pH 7.5. Phase separation run at 2°C, assays run at 25°C.

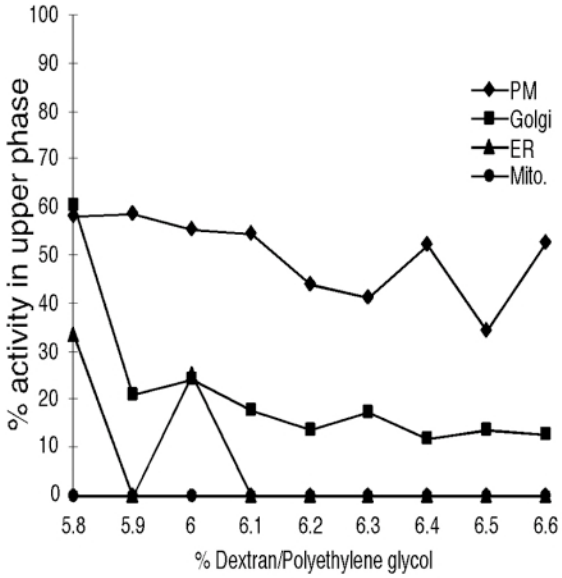

Figure 3. Effects of increasing polymer concentrations on the partition of different membranes in a microsomal fraction from *Chaetoceros gracilis*. Markers as in Figure 2. All other conditions as in Figure 2.

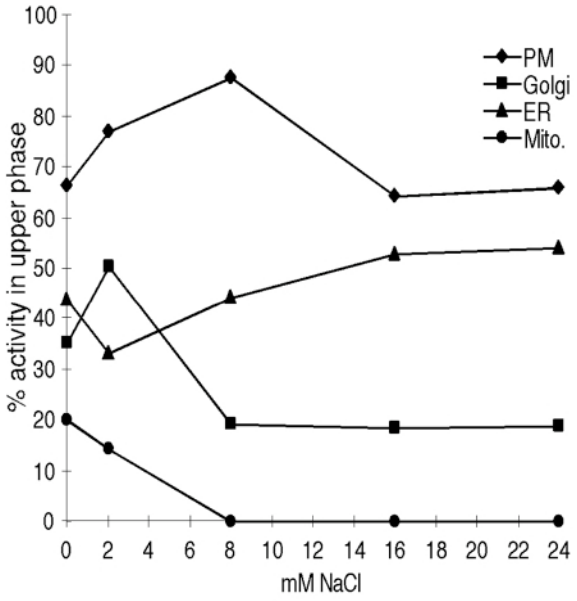

Figure 4. Effects of increasing polymer concentrations on the partition of different membranes in a microsomal fraction from *Isochrysis galbana*. Markers as in Figure 2. All other conditions as in Figure 2.

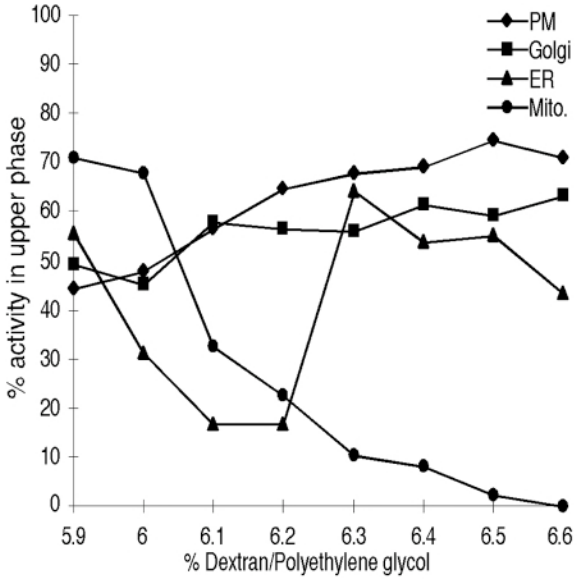

Figure 5. Effects of increasing NaCl concentrations on the partition of different membranes in a microsomal fraction from *Tetraselmis suecica*. Markers as in Figure 2. All other conditions as in Figure 2.

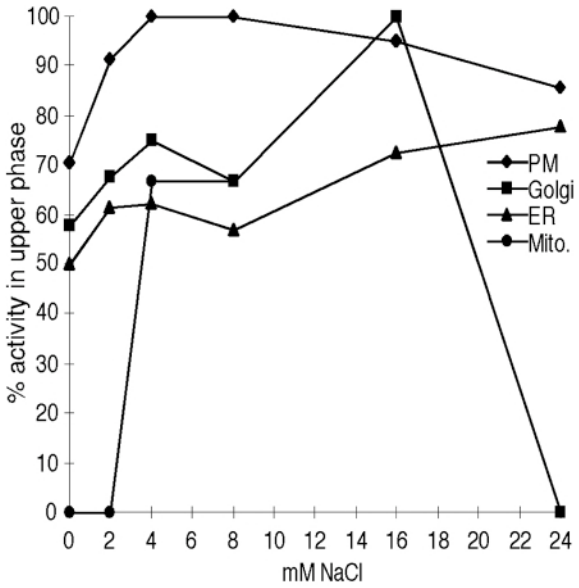

Figure 6. Effects of increasing NaCl concentrations on the partition of different membranes in a microsomal fraction from *Chaetoceros gracilis*. Markers as in Figure 2. The phase systems contained 6.5% Dextran, 6.5% PEG, 0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5, and 0-24 mM NaCl. Phase separation run at 2°C, assays run at 25°C.

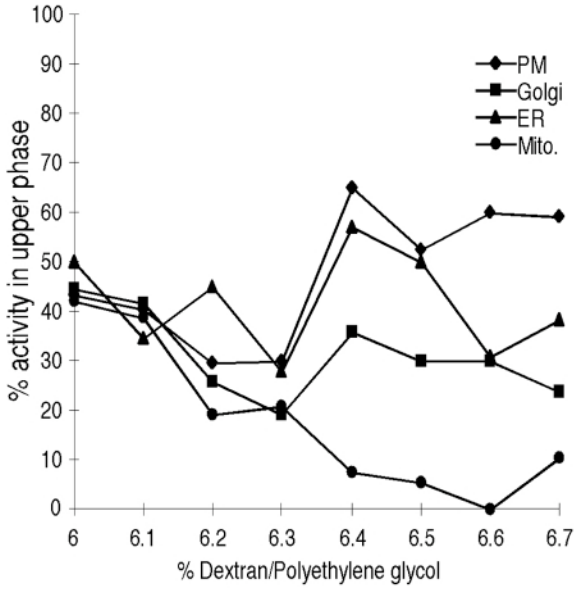

Figure 7. Effects of increasing NaCl concentrations on the partition of different membranes in a microsomal fraction from *Isochrysis galbana*. Markers as in Figure 2. The phase systems contained 6.4% Dextran, 6.4% PEG, 0.5 M sorbitol, 50 mM Tris-HCl, pH 7.5, and 0-32 mM NaCl. Phase separation run at 2°C, assays run at 25°C.