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Effects of Salinity on Chlorophyll Fluorescence from *Porphyra* Thalli and Comparison of Species with Different Intertidal Distribution

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Characteristic changes in chlorophyll fluorescence from thalli of red alga, *Porphyra*, under high salt stress and during subsequent recovery were investigated, and the differences in the sensitivity to the stress among four species of *Porphyra* with different intertidal distributions were compared. By the treatment of NaCl with 9% or higher concentrations, photochemical efficiency (Fv/Fm) decreased in a biphasic pattern: a rapid decrease was observed within 1-3 h and followed by a slow decline. The decrease of Fv/Fm was mainly due to the increase of Fo without significant increases of Fm. When the thalli treated with 15% NaCl for 6 h were returned to natural sea water for recovery, the increase of Fv/Fm also showed a biphasic pattern: a rapid increase of Fv/Fm was observed within 2 h and followed by a slow increase. Differences in the sensitivity to salt stress among the four species could be found during recovery after the treatment of severe salt stress. After the treatment of 20% NaCl for 6 h, Fv/Fm decreased below 0.3 in all of the four *Porphyra* species, and the species living in upper parts of the intertidal zone (*P. suborbiculata* and *P. pseudolinearis*) could recover better compared with the species in lower parts of the intertidal zone (*P. seriata* and *P. yezoensis*), during recovery for 24 h. The species collected from the coast of the South Sea seemed to be more tolerant than those in the East Sea.

Key words: Chlorophyll fluorescence assay, salinity, *Porphyra*, intertidal distribution, stress tolerance

Introduction

In natural habitats, the thalli of red alga, *Porphyra*, living in the intertidal zone lose their water content very fast up to 80~90% of its fresh weight when the tide is out. In the process of desiccation in the high light, the salinity of the remaining water on the surface of the thalli increase up to about 10 times higher than the salinity of natural sea water (Satoh et al., 1983). However, *Porphyra* in the intertidal zones are so well-adapted to this severe osmotic dehydration that they can fully recover their photosynthetic activities when rehydrated by incoming waves.

In the laboratory, it is not easy to make reproducible experiments with a similar speed and to the similar extent of dehydration as observed during the natural dehydration of *Porphyra* thalli. Therefore, some researchers investigated the effects of osmotic dehydration instead, and they reported several lines of evidence suggesting that air-drying and osmotic dehydration caused similar effects on algal tissues (Satoh et al., 1983; Smith and Berry, 1986; Smith et al., 1986). A gradual increase in salt concentration may reproduce gradual elevation effects of salt content on the surfaces of *Porphyra* thalli during the natural dehydration by air-drying in the intertidal zone.

The differences in the abilities of algae in the intertidal zone to tolerate dehydration and hyperosmotic stress are supposed to be related to the observed differences in algal distributions. Smith and Berry (1986) showed the relationship of limits of dehydration tolerance with the upper limits of

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vertical distribution in several intertidal and subtidal algal species. Similar correlation was also found among individuals of *P. perforata* with different intertidal distributions.

The physiological limitation which restricts subtidal and low tidal species from higher tidal areas is probably due to the damage on the primary processes of photosynthesis caused by severe dehydration (Satoh et al., 1983). Chlorophyll (Chl) fluorescence assay has been widely used as a sensitive and nondestructive technique to monitor changes in the photosynthetic apparatus of plants under environmental stress (Powels, 1984; Schreiber and Berry, 1977; Ögren, 1990; Lichtenthaler, 1988). This technique has been also applied to measure photosynthetic efficiencies of *Porphyra* under high light stress (Satoh and Fork, 1983; Sibbald and Vidaver, 1987; Bose et al., 1988) and under high salt stress (Satoh et al., 1983).

In spite of these intensive research on the photosynthetic machinery of *Porphyra* under various stress conditions, no intensive effort has been reported on the comparison of the tolerances against osmotic dehydration among various species of *Porphyra*, and our knowledge about the tolerance mechanism is very limited. In this paper, we investigated the characteristic changes in photosynthetic apparatus of *Porphyra* under high salt stress, and compare the differences in the sensitivity to osmotic dehydration among the four species of *Porphyra* growing in different natural habitats using Chl fluorescence assay.

Materials and Methods

Collection of samples

Fresh thalli of *Porphyra* were collected at Masanri, Pohang, Kyungsangbukdo in the coast of the East Sea (*P. pseudolinearis* and *P. yezoensis*) and at Mibeop, Hadonggun, Kyungsangnamdo in the coast of the South Sea (*P. suborbiculata* and *P. seriata*) in Korea. At each location, two species of *Porphyra* living in different parts of the intertidal zone were collected just after the tide was out in January of 1997. For the comparison of the differences in their tolerance against salt stress, *P. pseudolinearis* and *P. surbobiculata* were collected at the upper limit in their habitats, and *P. yezoensis* and *P. seriata* were collected in the middle and at the relatively lower limit of their habitat, respectively. The collected thalli were kept in natural sea water with air-bubbling at 4℃ in the dim light. The natural sea water was stored at 4℃ and was sterilized by membrane (pore size 0.2 μm) filtration before use. At least two days before the start of an experiment, thalli were acclimated at 18℃ in the light with a photon flux density (PFD) of 1 μmol m^{-2} s^{-1}.

Treatment of salt stress and the recovery in natural sea water

For salt treatment, thalli were cut into about 2 cm×2 cm segments and were incubated in the natural sea water added with various concentrations (0%, 3%, 6%, 9%, 12%, 15% and 20%) of NaCl in the light with a PFD of 1 μmol m^{-2} s^{-1} for 6 h or 24 h. The salinity of natural sea water was approximately 3.4%. The concentration of NaCl mentioned in this paper is the concentration of NaCl added to the natural sea water. For their recovery, the salt-treated thalli were washed once with natural sea water and incubated in natural sea water at 18℃ in the light with a PFD of 1 μmol m^{-2} s^{-1}.

Measurement of chlorophyll fluorescence

Chl fluorescence was measured using a portable fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., England) as described in Eu et al. (1996). The thalli were adapted in the dark for 2 min before Chl fluorescence was monitored. The maximum variable fluorescence (Fv) was obtained by subtraction of the initial Chl fluorescence (Fo) from the maximum yield of fluorescence (Fm). The ratio of Fv/Fm was used to show the potential quantum yield of photochemical reactions in photosynthesis or photochemical efficiency in short (Horton and Bowyer, 1990).

Results

Effects of salt stress on the photosynthetic efficiencies of *Porphyra* thalli

The effects of salt stress were dependent on the concentration of NaCl as shown in Fig. 1. In all of the four species of *Porphyra*, photochemical efficiency did not decrease significantly in thalli treated with 3% and 6% NaCl. At concentrations from 9% to 20%, a rapid drop of Fv/Fm was observed within 1-3 h and followed by a slow decline.

The decrease of Fv/Fm was mainly due to the increase of Fo. Fig. 2 shows an example in

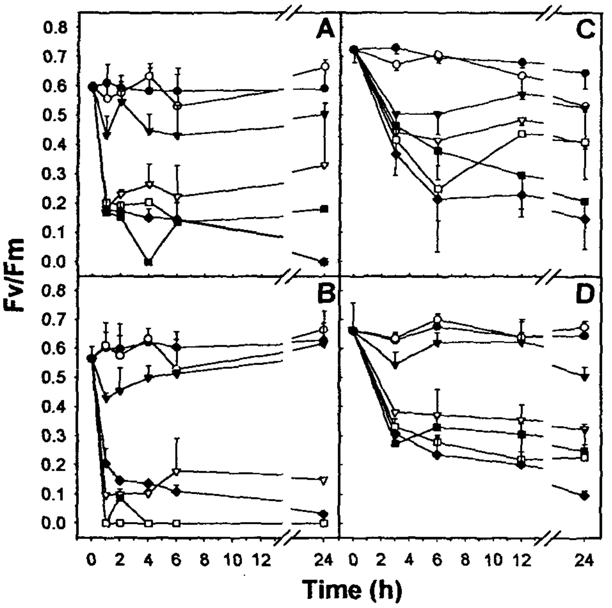

Fig. 1. Effects of salinity on Fv/Fm of thalli in the four species of *Porphyra*.
About 2 cm×2 cm thalli segments were incubated in natural sea water treated with 0% (●), 3% (○), 6% (▼), 9% (▽), 12% (■), 15% (□) and 20% (◆) NaCl. A *Porphyra seriata*, B *Porphyra suborbiculata*, C *Porphyra pseudolinearis* and D *Porphyra yezoensis*. Results are mean plus or minus S.D. n=4 (or less in cases with very low Fv/Fm).

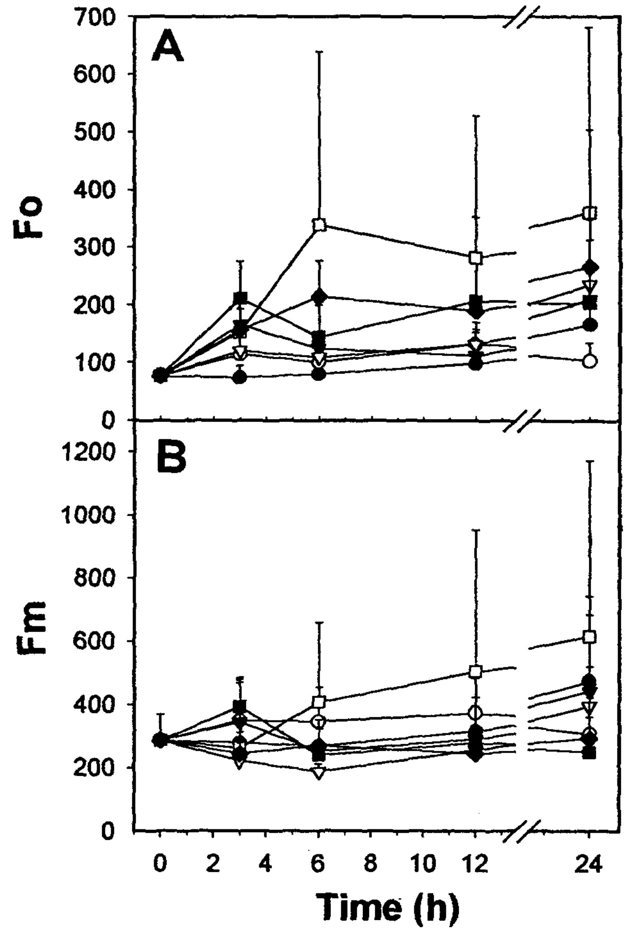

Fig. 2. Effects of salinity on Fo (A) and Fm (B) of thalli in *Porphyra pseudolinearis*.
About 2 cm×2 cm thalli segments were incubated in natural sea water treated with 0% (●), 3% (○), 6% (▼), 9% (▽), 12% (■), 15% (□) and 20% (◆) NaCl.
Results are mean plus or minus S.D. n=4 (or less in cases with very low Fo or Fm).

P. pseudolinearis, where Fo increased significantly (Fig. 2A) without corresponding increases of Fm (Fig. 2B). The changes in Fo seemed to be biphasic in parallel with the changes in Fm. Similar changes in Chl fluorescence parameters were also observed in all the other three species of *Porphyra* (data not shown).

Recovery of the reduced photosynthetic efficiencies of *Porphyra* thalli in natural sea water

Because the rapid phase of the decrease of Fv/Fm ended within 3 h, and a significant water loss from the thalli was expected to be completed during this time, the thalli were kept in 15% NaCl for 6 h and were returned to natural sea water for recovery. As shown in Fig. 3, the increase of Fv/Fm also showed a biphasic pattern. A rapid increase of Fv/Fm was observed within 2 h and followed by a slow increase. The increase of Fv/Fm during recovery was accompanied by a decrease of Fo in parallel (data not shown).

Differences in sensitivities to salt stress among the four species of *Porphyra*

To see if there are any differences in sensitivities to salt stress among different species living in different habitats, the changes in Chl fluorescence from thalli of each *Porphyra* species was compared after the salt treatment for 6 h (Fig. 4). In *P. suborbiculata* and *P. seriata*, the concentration dependent reduction in Fv/Fm was more prominent than in the cases of the other two species in the range of added salt concentrations from 6% to 9%. However, the extent of the reduction in Fv/Fm by high salt treatment does not seem to reveal their sensitivities to salt stress, because the recovery of Fv/Fm in *P. suborbiculata* was to the highest value among the four species compared (Fig. 3).

In Fig. 3, both *P. suborbiculata* and *P. seriata* could recover completely during the recovery period for 24 h, although the two species were living in different tidal levels in the intertidal zones. To see if we can observe any differences in the reversibility

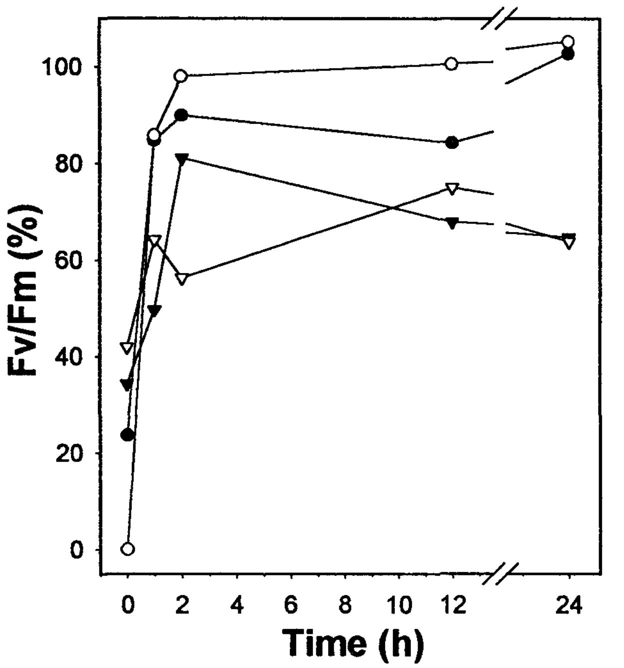

Fig. 3. Recovery of Fv/Fm of thalli after the treatment of 15% NaCl for 6 h in four species of *Porphyra*. *Porphyra seriata* (●), *Porphyra suborbiculata* (○), *Porphyra pseudolinearis* (▼), and *Porphyra yezoensis* (▽).

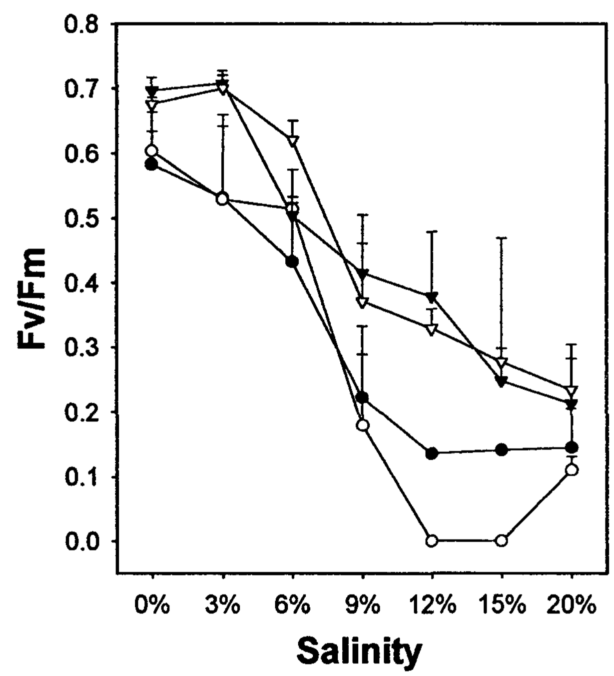

Fig. 4. Changes in Fv/Fm of thalli after the treatment of various concentrations of NaCl for 6 h in four species of *Porphyra*. *Porphyra seriata* (●), *Porphyra suborbiculata* (○), *Porphyra pseudolinearis* (▼), and *Porphyra yezoensis* (▽). Results are mean plus or minus S.D. n=4 (or less in cases with very low Fv/Fm).

as it can be expected from the differences in their natural habitats, the recovery of photosynthetic efficiencies were monitored after the treatment of 20 % NaCl for 6 h and 24 h as shown in Figs. 5 and 6, respectively. Although the changes in Fv/Fm during recovery of four species did not show same patterns in the two Figures, the species living in upper parts of the intertidal zones (*P. suborbiculata* and *P. pseudolinearis*) could recover well compared with the species in lower parts of the intertidal zones (*P. seriata* and *P. yezoensis*).

Discussion

The results of the present study demonstrated that the photosynthetic apparatus in thalli of four species of *Porphyra* growing in intertidal zones seemed to adapt very rapidly to osmotic dehydration, and Chl fluorescence assay can be used to determine the differences in the sensitivity to salt stress among the four species of *Porphyra* growing in different habitats.

Under high salinity stress, the reduction pattern of photosynthetic efficiency or Fv/Fm was biphasic (Fig. 2), which was mainly due to a noticeable increase of Fo (Fig. 3A). During recovery in natural sea water, this process was reversed and also showed a biphasic pattern (Figs. 4, 6 and 7). The water flux in and out of the algal tissue to the changes in salinity is also known to show biphasic patterns (Kirst, 1990). The first phase reflects a rapid changes in turgor pressure caused by massive water flux in or out of thalli following the osmotic gradient. The second phase is rather slow accompanied by osmotic adjustments of osmolytes. Therefore, the rapid phase of the changes in Fv/Fm and Fo observed in this study during osmotic dehydration and subsequent rehydration seems to reflect the rapid water flux in or out of thalli.

An increase of Fo, a decrease of Fv/Fm and/or a decrease of Fm/Fo are generally observed symptoms in plant leaves under various environmental stress. Under severe dehydration in corn leaves, photosynthetic apparatus was found to be irreversibly damaged when an increase of Fo was observed (Xu et al., 1998). From the decrease of Fv/Fm below about 0.2 as observed in Fig. 1 or two to three fold increase of Fo as shown in Fig. 2A, the cells might be thought as being irreversibly damaged in the cases of higher plants under stress.

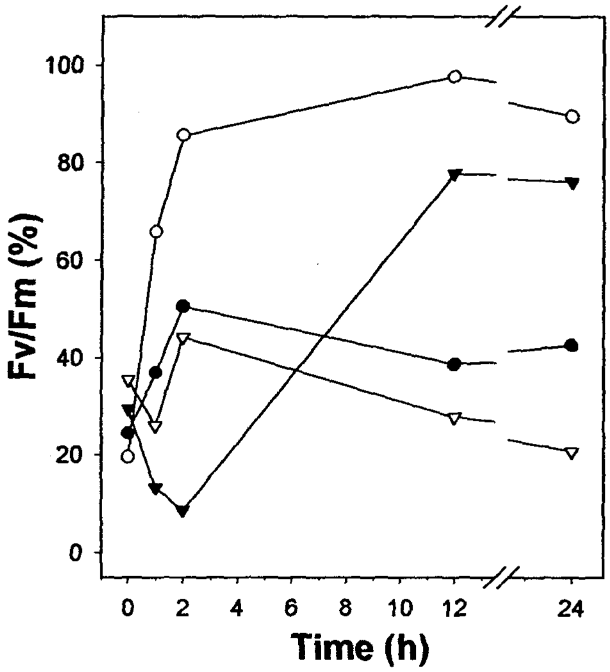

Fig. 5. Recovery of Fv/Fm of thalli after the treatment of 20% NaCl for 6 h in four species of Porphyra.
Porphyra seriata (●), *Porphyra suborbiculata* (○), *Porphyra pseudolinearis* (▼), and *Porphyra yezoensis* (▽).

However, it was not always true in the cases of *Porphyra* species as shown in Figs. 3, 5 and 6, indicating that species of *Porphyra* growing in the intertidal zones had exceptionally strong resistance to high salinity and severe dehydration stress.

Although a noticeable changes in Fo in *Porphyra* thalli was observed under high salinity stress in the low light, Satoh et al. (1983) reported an increase of O-I level in the fluorescence rise curve in *P. perforata* under high salt stress in the light. In barley, Belkhodja et al. (1994) also reported an increase of O-I level, when the leaves were incubated in a high salinity solution in the light. However, Blackwell and Gilmour (1991) reported an increase of Fo in unicellular green algae Chlorococcum submarinum under high salinity stress. Our detailed examination of the rise kinetics of the Chl fluorescence induction curve also confirmed our observation.

The osmotic dehydration allows us to replicate the dehydration of *Porphyra* thalli in natural habitats with greater reproducibility than air-drying does. The test of osmotic dehydration performed on this purpose is based on several experimental results suggesting that the two processes had similar effects on algal tissue (e.g. Smith and Berry, 1986; Smith et al., 1986). This kind of experiment is also based on the assumption of the impermeability of algal tissue to NaCl and the existence of possible common mechanisms for the resistance to two different-but-closely linked stress, which are remained to be confirmed.

In higher plants, the mechanism of rapid dehydration is different from that of long-term drought, and the detailed examples are listed in Ö gren (1990). The rapid water flux during high salt stress and subsequent recovery suggests that the process observed in *Porphyra* may be similar to the rapid dehydration. However, the major characteristics of *Porphyra* to salt stress are the fast and reversible recovery in terms of Chl fluorescence parameters, especially a unique reversible changes in Fo, which is not frequently found in higher plants. Although the rapid phase observed in this study during osmotic dehydration and subsequent rehydration seems to include the rapid water flux in or out of thalli, we would rather assume that the changes in Fo is a kind of adaptation mechanism in *Porphyra* species showing their tolerance against high salinity stress. Because most of the changes in Fo was completed within 10 min (data not shown), we can expect that this process involves physicochemical processes, and the changes in Fo proceeds rather fast to assume the involvement of genetic control. Understanding the mechanism for the changes in Fo under high salt stress will be very important in order to reveal the adaptation mechanism of *Porphyra* against severe dehydration and high salt stress.

An approach to understand the tolerance mechanism is to compare the physiological characteristics of several species with different sensitivities to salinity stress. For this purpose, we collected the four species of *Porphyra*, two from the coast of the East Sea and two from the coast of the South Sea. There was no significant differences among the four species in their sensitivities to salt stress which could be correlated well with their natural habitats (Fig. 4). Instead, *P. suborbiculata* could recover to the highest value among the four species compared (Fig. 3), although the reduction in Fv/Fm in higher salt concentrations was the most sensitive in *P. suborbiculata* (Fig. 4). Therefore, the ability to keep high Fv/Fm values may be correlated well with the resistance to severe stresses in many cases of terrestrial plants, but it may not be true in the case of *Porphyra*.

Differences in the sensitivity to salt stress among the four species of *Porphyra* could be found during recovery after the treatment of severe salt stress. After the treatment of 20% NaCl for 6 h, Fv/Fm decreased below 0.3 in all of the four *Porphyra* species (Fig. 4). During recovery after the stress treatment for 6 h or 24 h, the species living in upper parts of the intertidal zones (*P. suborbiculata* and *P. pseudolinearis*) could recover better compared with the species in lower parts of the intertidal zones (*P. seriata* and *P. yezoensis*). Smith et al. (1986) could also find differences in the sensitivity to high salt and air-drying among several intertidal and subtidal species of *Porphyra* from the changes in the cell morphology and 77K fluorescence spectra during recovery after the stress treatment. Smith and Berry (1986) also compared differences in the sensitivity to high temperature and high salt stress among several intertidal and subtidal algal species from the ability to recover photosynthetic oxygen evolving rates during recovery after the stress treatment.

Although Figs. 6 and 7 shows the correlation of the stress tolerance with the habitats of *Porphyra* in the intertidal zones, the changes in Fv/Fm during recovery of the four species did not show same patterns in the two Figs. Interestingly, the two species collected from the coast of the South Sea (*P. suborbiculata* and *P. seriata*) could recover better than the other two species (*P. pseudolinearis* and *P. yezoensis*) found in the East Sea after the samples were treated with 20% NaCl for 24 h (Fig. 6). A possible reason for this difference is that the tidal range is longer in the coast of the South Sea than in the coast of the East Sea. In less severe stress conditions, or after the treatment of 20% NaCl for 6 h, both of the high tidal species could recover better than the low tidal species (Fig. 5). Although some of these suggestions should be confirmed with repeated experiments using many different algal species, these results showed that Chl fluorescence assay can be used to determine the differences in the sensitivity to salt stress among species of *Porphyra* growing in different locations in the intertidal or subtidal zones and among species growing in the similar tidal zones but in different geographic regions. Regarding to this kind of comparison, one thing should be mentioned is that the experiments should be repeated at the similar time in the season, because the stress tolerance might vary seasonally.

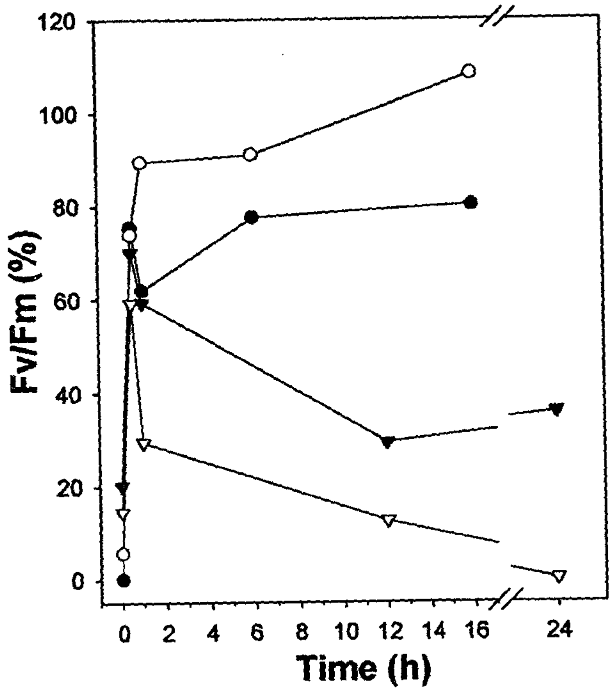

Fig. 6. Recovery of Fv/Fm of thalli after the treatment of 20% NaCl for 24 h in four species of Porphyra. *Porphyra seriata* (●), *Porphyra suborbiculata* (○), *Porphyra pseudolinearis* (▼), and *Porphyra yezoensis* (▽).

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