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NOTE

Release of mannitol from *Pilayella littoralis* (Phaeophyta: Ectocarpales) in response to hypoosmotic stress

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ABSTRACT: Rapid release of mannitol was observed when plants of the euryhaline marine alga *Pilayella littoralis* (L.) Kjellm. were transferred from a saline medium (either 100 % or 200 % sea water) to freshwater. Mannitol loss varied as a direct function of the pre-treatment temperature and the temperature of the freshwater incubation medium, with maximum release of mannitol (22.9 % of the total intracellular pool) at 25 °C. The release of this low-molecular-weight organic osmoticum has important consequences for osmotic adjustment in *Pilayella littoralis* and may act as a source of fixed carbon for associated heterotrophic microorganisms.

Members of the Phaeophyta are major contributors to the total primary productivity of numerous marine intertidal habitats (Littler & Murray 1974). The release of dissolved organic carbon (DOC) from these plants has been the subject of several recent studies, due to the potential significance of such release to (i) measurements of primary production based on standing crop and (ii) carbon flow in coastal marine environments. Early studies (Khailov & Burkalova 1969, Sieburth 1969) suggested that 30 % or more of the total carbon fixed by marine brown algae could be released by the plants. However, subsequent investigations have given somewhat lower values (1 to 4 %) for the release of DOC from whole, healthy plants (e.g. Brylinski 1977), and Moebus & Johnson (1974) have suggested that the earlier, higher values may have been due in part to tissue wounding during the preparation of small portions of plant material for experimental purposes. Intact, undamaged thalli of the intertidal marine green alga *Enteromorpha prolifera* also released minimal amounts of DOC (1 to 6 %) during incubation in sea water (Pregnall 1983).

The majority of the DOC released by these macroalgae is assumed to be in the form of small molecules and polysaccharides (Hellebust 1974), which would be readily utilized by associated heterotrophic microbes (Williams & Yentsch 1976) and may thus represent an important source of carbon to heterotrophic marine organisms.

It is also recognised that the physical and climatic stresses imposed on marine macroalgae during periods of emersion may increase DOC release. Sieburth (1969) has shown that laboratory simulation of a period of rainfall can increase the rate of DOC release by up to 300 % in *Fucus vesiculosus* and 100 % in *Ascophyllum nodosum*. Rehydration of thalli following periods of desiccation may also increase DOC release (Sieburth 1969, Moebus et al. 1974).

In the experiments described above, the nature of the liberated compounds has not been considered, or was resolved in general terms (e.g. carbohydrates, phenolics, proteins). In contrast, experimental studies of the effects of salinity stress (both hypoosmotic and hyperosmotic shock treatment) on microalgae have shown that a major component of the carbon released can be attributed to the loss of their internal organic solutes. The liberation of intracellular low-molecular-weight metabolites from hypoosmotically-stressed cells has been demonstrated for several microalgae, including *Monochrysis lutheri*, which released cyclohexanetetrol (see Hellebust 1974), *Platymonas subcordiformis*, which released mannitol (Kirst 1977), *Phaeodactylum tricornutum*, which liberated proline (Schobert 1980) and the filamentous, colonial blue-green alga (cyanobacterium) *Rivularia atra*, which released trehalose (Reed & Stewart 1983). While data exist to show that marine brown macroalgae accumulate the hexitol mannitol as an osmotically-active intracellular solute (see Reed et al. 1985a), there is a lack of information on the extracellular liberation of mannitol upon osmotic shock treatment. The present study was carried out to assess the effects of hypoosmotic shock upon mannitol release from plants of the euryhaline brown alga *Pilayella littoralis* (L.) Kjellm

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an alga found in estuarine and marine intertidal habitats where rapid changes in salinity may occur.

Pilayella littoralis was collected in June 1985 from an epiphytic population growing on *Fucus vesiculosus* at Fife Ness, Scotland. Plants were maintained in the laboratory in media based on ASP12S as described previously (Reed et al. 1980), prepared at a salinity corresponding to 100% sea water (≡35‰) or 200% sea water (≡70‰), pH 8.0. Plant material was incubated for 24 h in each medium, at a temperature of 15°C and under continuous illumination (40 μmol m^{-2} s^{-1}) prior to experimentation. Before subjecting the plants to hypoosmotic stress, they were incubated for a further period of 1 h at either 5, 15 or 25°C. Plant tissue was freed of adhering medium by vacuum filtration (Reed & Barron 1983) and then transferred to 50 cm^3 of distilled water with added $NaHCO_3$ at 2 mol m^{-3} to raise the pH to 8.0 (freshwater medium) at a temperature of either 5, 15 or 25°C. Thalli were separated from this freshwater medium after a 2 min incubation period, using vacuum filtration. Samples of bathing medium and plant tissue were frozen rapidly using liquid nitrogen and then lyophilized. Identification and quantification of mannitol was carried out, using gas-liquid chromatography of trimethyl-silyl derivatives with arabitol as internal standard, as described in detail by Reed & Davison (1984).

The loss of mannitol from plants transferred from 100% sea water to freshwater is shown in Table 1. Mannitol release was observed in all treatments. Mannitol loss was dependent upon the pre-treatment temperature, with plants from 25°C showing the greatest overall liberation of this hexitol upon hypoosmotic shock treatment. The release of mannitol was also dependent upon the temperature of the freshwater medium, increasing by 3 to 5-fold between 5 and 25°C. Maximum loss of internal mannitol was thus observed in plants pre-treated at 25°C and subjected to hypoosmotic shock at 25°C.

Plants kept in a hypersaline medium (200% sea water) for 24 h increased their intracellular mannitol level by approximately 50% (Table 2) and hypoosmotic shock treatment led to greater losses of mannitol, in both absolute and proportional terms, at all temperatures (cf. Table 1). However, similar effects of temperature were observed, with increasing mannitol release as a direct function of (i) the pre-treatment temperature and (ii) the temperature of the freshwater medium. Loss of mannitol from *Pilayella littoralis* kept at 25°C throughout the experimental procedure was in excess of one-fifth of the total internal pool, while plants kept at 5°C liberated less than one-tenth of their intracellular hexitol content. Thus cold shock did not increase the loss of intracellular metabolites, in contrast to the

Table 1. Release of mannitol from *Pilayella littoralis* transferred from full-strength (100%) sea water to freshwater at pH 8.0 (3 replicates per treatment: mean ± SD). Percentage loss of internal mannitol is shown in parentheses

Initial temperature (°C)	Internal mannitol (g kg^{-1})	Mannitol release (g kg^{-1} dry wt) after 2 min at: 5°C	15°C	25°C
5	37.35 ± 3.16	0.72 ± 0.08 (1.9)	1.92 ± 0.13 (5.1)	3.81 ± 0.27 (10.2)
15	37.11 ± 4.56	1.15 ± 0.03 (3.1)	2.14 ± 0.06 (5.8)	3.34 ± 0.25 (9.0)
25	38.85 ± 0.11	2.23 ± 0.11 (5.7)	3.45 ± 0.54 (8.9)	6.06 ± 0.61 (15.6)

Table 2. Release of mannitol from *Pilayella littoralis* transferred from hypersaline conditions (200% sea water) to freshwater at pH 8.0 (3 replicates per treatment: mean ± SD). Percentage loss of internal mannitol is shown in parentheses

Initial temperature (°C)	Internal mannitol (g kg^{-1})	Mannitol release (g kg^{-1} dry wt) after 2 min at: 5°C	15°C	25°C
5	54.47 ± 5.42	3.23 ± 0.56 (5.9)	7.80 ± 1.12 (14.3)	10.73 ± 0.54 (19.7)
15	53.36 ± 4.31	4.23 ± 0.61 (7.9)	7.20 ± 0.77 (13.5)	10.02 ± 0.32 (18.8)
25	59.30 ± 2.15	4.69 ± 0.76 (7.9)	9.55 ± 0.38 (16.1)	13.58 ± 1.79 (22.9)

observed loss of cell solutes upon cold shock treatment in the blue-green alga *Anacystis nidulans* (Ono & Murata 1981).

The mortal stain Evans' Blue was used to check the integrity of plants subjected to hypoosmotic shock (Reed & Barron 1983), showing that the cells remained intact upon transfer to freshwater medium. There was no evidence of increased mortality due to lysis or bursting of the cells in response to increased turgor. Furthermore, a time-course for mannitol release in plants transferred from 200% sea water to freshwater showed that release occurred within a 2 min period following hypoosmotic shock, with little indication of any further loss over the remaining period, up to 15 min (Fig. 1). No mannitol release was observed from

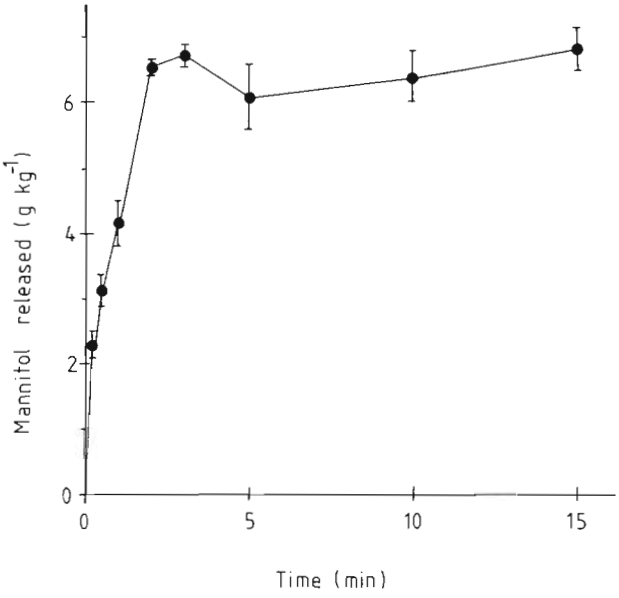

Fig. 1. Time course of mannitol release from *Pilayella littoralis* transferred from 100% sea water (ASP12S medium) to freshwater at 25°C and pH 8.0 (3 replicates per treatment: mean ± SD)

plants *maintained* in either 100 or 200% sea water, indicating that hypoosmotic stress treatment was responsible for the observed release of mannitol.

Taken together, these results are consistent with the hypothesis that a transient change in the permeability of the plasma membrane of *Pilayella littoralis* may have been induced by hypoosmotic stress, leading to a loss of intracellular metabolites. Similar phenomena are believed to be responsible for the loss of internal osmotica from microalgal cells (Kirst 1977, Reed et al. 1985b).

The present study supports previous observations that DOC release from marine macroalgae may be increased by periods of simulated rainfall (Sieburth 1969, Pregnall 1983), although the values in Tables 1 and 2 are higher than those obtained by Sieburth (1969) for *Fucus vesiculosus* and *Ascophyllum nodosum*. This may be due in part to differences in morphology. *Pilayella littoralis* is a filamentous alga, while *F. vesiculosus* and *A. nodosum* have parenchymatous thalli. All of the cells of *P. littoralis* can thus be exposed to very rapid changes in salinity since the organisation of the thallus permits rapid penetration of the hypoosmotic medium under experimental conditions. The present study also suggests that the effects of rainfall on *P. littoralis* will be modified by the ambient temperature and that greater losses of mannitol may occur during periods of rainfall in the summer months.

The release of mannitol can be considered as a means of reducing the intracellular osmotic pressure and therefore lowering cell turgor (Kirst 1977). However, loss of fixed carbon due to hypoosmotic shock treatment would be energetically more wasteful than conversion to laminaran, the polysaccharide storage compound of brown algae. The blue-green alga *Rivularia atra* released approximately 10 to 12% of the total intracellular pool of the disaccharide trehalose upon transfer from 100% sea water to freshwater at 20°C, while the remainder was dissimilated, presumably by conversion to glycogen, thus lowering the osmotic pressure within the cell (Reed & Stewart 1983). It seems probable that solute leakage, due to uncontrolled, temporary changes in membrane permeability is common to osmotically-stressed eukaryotic (Reed 1984) and prokaryotic algae (Reed et al. 1985b).

In natural environments, *Pilayella littoralis* may be subjected to rapid salinity changes due to freshwater run-off and/or rainfall. Any mannitol that is released as a result of rapid 'downshock' is likely to be an easily-assimilated source of organic cabon for heterotrophic bacteria since (i) specific permease systems exist to enable these bacteria to accumulate mannitol (Jacobson et al. 1983), (ii) it is a compound that may be converted easily to key metabolic intermediates, e.g. fructose and (iii) a single low-molecular-weight compound may be easier to accumulate and utilize than a complex mixture of carbon compounds. The laboratory culture of heterotrophic marine bacteria using low-molecular-weight solutes released from algal cells (Nalewajko 1977) is also consistent with this hypothesis.

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