



Short Communication

# Transglutaminase activity decrease during acclimation to hyposaline conditions in marine seaweed *Grateloupia doryphora* (Rhodophyta, Halymeniaceae)

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## KEYWORDS

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## Summary

Polyamines (PAs), such as diamine putrescine (PUT), triamine spermidine (SPD) and tetraamine spermine (SPM) have been related to environmental stress, including salt stress. A marine red macrophyte alga *Grateloupia doryphora* (Montagne) Howe was used to investigate the role of PAs during acclimation to moderate hyposaline conditions (incubation 24 h in 18 psu seawater as compared to 36 psu of natural seawater). The results obtained showed that a moderate hyposaline shock caused an increase in the free fraction of PUT, SPD and SPM, mainly due to a decrease in TGase activity, together with an apparent increase in the L-arginine dependent PAs synthesis (ODC and arginase decreased, and ADC slightly increased). The photosynthetic rate increased in thalli when exposed to free SPD at 18 psu, but it did not increase at 36 psu.

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## Introduction

The preservation of important cell structures has been assumed to be the result of polyamine (PA)

accumulation during salt stress in several higher plants (Erdei et al., 1990, 1996). Aliphatic amines, like diamine putrescine (PUT), triamine spermidine (SPD) and tetraamine spermine (SPM), commonly ascribed as PAs may be found in marine algae in common free and bound compartments (Marián et al., 2000). In spite of intertidal marine algae daily facing salinity fluctuations, reports relating changes in PA contents and their enzyme activities to salinity changes are relatively scarce (Lee, 1998; Lee and Chen, 1998).

*Abbreviations:* ADC, L-arginine decarboxylase; ODC, L-ornithine decarboxylase; PAs, polyamines; PUT, putrescine; SPD, spermidine; SPM, spermine; TGase, transglutaminase.

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The differences in the PA endogenous levels encountered, as related to physiological events, like salt stress, are determined by *de novo* synthesis, along with the mobilisation between free and bound endogenous PA compartments (Davis et al., 1992). The synthesis of SPD and SPM starts at PUT, which is synthesized by L-ornithine decarboxylase (ODC, EC. 4.1.1.17), or L-arginine decarboxylase (ADC, EC.4.1.1.19). The enzyme arginase (EC. 3.5.3.1) converts L-arginine into L-ornithine. Transglutaminases (TGases, EC.2.3.2.13) may join free PAs to the  $\gamma$ -carboxamide moiety of two (*bis*) glutamine residues of proteins, thus contributing to the bound fraction of PAs in plants (Del Duca et al., 1997; Della Mea et al., 2004).

This work deals with potential changes that moderate hyposaline shocks (18 psu) may promote in the endogenous contents of free and conjugated PAs. To determine whether this situation may benefit the acclimation of the alga, we investigated (i) whether physiological performance might be affected by the changes in PAs; thus, we controlled the effects of PAs on photosynthesis and (ii) whether it was the result of regulation of the metabolism of PAs through related enzymatic activities.

## Materials and methods

The species used in this study, *Grateloupia doryphora* (Montagne) Howe, is an intertidal red alga living in the tide pools along the northeast coast of Gran Canaria (Canary Islands). Within 2 h after collection, infertile axes (ca. 4 g FW,  $n = 3$ ) were cultured for 24 h in 500 mL aerated glass vessels with 300 mL of autoclaved seawater at 36 psu or diluted with Milli-Q (A10 gradient) grade water (Millipore) to reach 18 psu as a hyposaline shock at  $20 \pm 2$  °C and  $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in a growth chamber.

PAs were extracted, dansylated and quantified by HPLC as previously reported (Sacramento et al., 2004). ODC and ADC enzyme activities were determined in the crude extract from thalli as described elsewhere (Sacramento et al., 2004). Arginase enzyme activity was determined by the spectrophotometric quantification of its product L-ornithine, as described by Primikiriou and Roubelakis-Angelakis (1999). To quantify TGase, the crude extract of each sample was precipitated with 70% ammonium sulphate and desalted through G25. A volume of 250  $\mu\text{L}$  was pre-incubated with 10 mM  $\text{CaCl}_2$  for 15 min at RT, then mixed with

250  $\mu\text{L}$  of an endogenous protein substrate (EPS) prepared by concentrating and desalting the protein in the crude extract of *Grateloupia*, 140  $\mu\text{L}$  of substrate cocktail (100  $\mu\text{L}$  of 0.2 mM SPD from Sigma Co., and 40  $\mu\text{L}$  of [ $^{14}\text{C}$ ]-SPD,  $1.85 \text{ MBq mL}^{-1}$   $4.14 \text{ GBq mmol}^{-1}$  from Amersham Biosciences, UK), final reaction volume was adjusted to 1 mL with phosphate buffer (70 mM, pH 7). Counts obtained were always corrected with two controls that assessed TGase in EPS and unspecific non-enzymatic linked SPD. TGase activity was determined after 2 h at 37 °C, though the reaction was seen to be linear for 3 h. Some samples served to check the presence of  $\gamma$ -glutamyl-SPD residues as an evidence of present transglutaminase activity. In these, the final pellet was redissolved in 100  $\mu\text{L}$  of buffer (Tris-glycine pH 8.8, 2% SDS) instead of scintillant, to count for bound SPD, and loaded on denatured SDS electrophoresis (Bio-Rad miniprotein-III electrophoresis system) in acrylamide gels (12% T) in Tris-HCl 3 M pH 8.8, and Tris-glycine buffer pH 8.8 as a reservoir buffer. After electrophoresis, acrylamide gel was washed with distilled water and protein bands were diced into approximately 1 mm. Proteins were extracted, following the procedures for protein gel digestion with several proteases (Havlis et al., 2003). The amount of SPD was compared before and after HCl digestion of protease extract, and the significant difference observed was assumed to come from SPD liberation from mono or *bis*  $\gamma$ -glutamyl-SPD ( $1.7 \pm 0.85$  and  $6.85 \pm 3.35 \mu\text{M}$  SPD in samples before and after HCl hydrolysis, respectively).

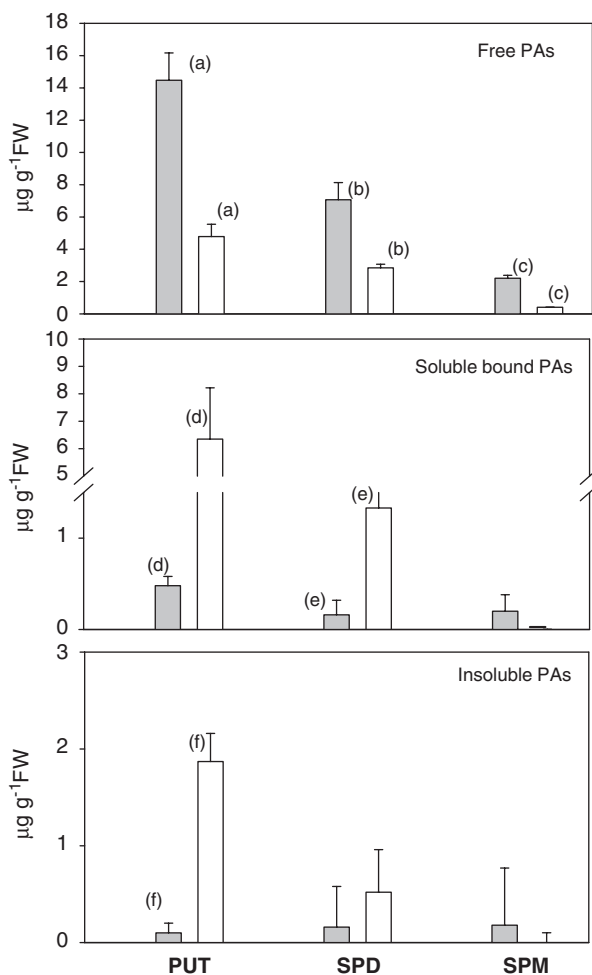
Photosynthetic activity was measured with explants incubated for 1 h in 36 or 18 psu seawater, in the presence or absence of  $10^{-6}$  M of PUT, SPD or SPM. The maximum photosynthetic rate was recorded at saturating  $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in a Clark-type electrode system (Hansatech, Norfolk, UK) as previously described (Rodrigo and Robaina, 1997).

Statistical analysis was performed with SPSS 13.0 (SPSS Inc., Chicago, USA). Data were analysed by non-parametric Kolmogorov-Smirnov tests to detect differences between treatments.

## Results and discussion

Lee and Chen (1998) reported PUT accumulation in the green alga, *Ulva fasciata*, and Lee (1998) concluded that this PUT increase was the causal factor of severe hyposaline injury in this, and another six marine species. As seen in Fig. 1,

the entire free fraction of PUT, SPD and SPM accumulated under hyposaline conditions, together with a decrease in soluble-bound and insoluble PUT, and soluble-bound SPD. The bound and insoluble SPM were apparently invariable. Our red alga appears to be of the species type which preferentially modifies the free fraction of PAs in its reaction to stress conditions. In contrast to the results obtained by Lee (1998), our results are related to moderate hyposaline conditions (*Grateloupia* is able to grow and regenerate if cultivated in such conditions, Robaina et al., 1990), and, therefore, to physiological events that caused the algae to adapt to toleration levels. As an example, benefits in photosynthetic activity could be ob-

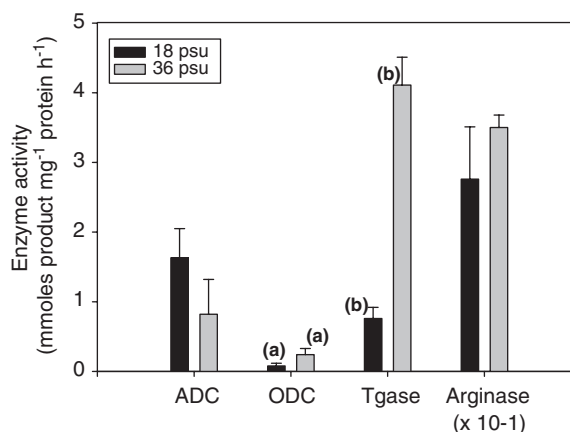


**Figure 1.** Endogenous levels of PUT, SPD and SPM ( $\mu\text{g g}^{-1}\text{FW}$ ) in all fractions of thalli incubated for 24h under hyposaline shock (18psu. Dark bars) as compared to thalli in natural salinity of seawater (36psu. Open bars). Data are the mean  $\pm$  SE of  $n = 4-6$  samples. The same letter denotes significant differences between treatments ( $P < 0.05$ ).

tained from free PA accumulation, since thalli subjected to hyposaline treatment showed a general trend to increase their  $P_{\text{max}}$  values when incubated with the PUT, SPD or SPM, reaching their highest significant differences in  $P_{\text{max}}$  when the explants were incubated in the presence of  $10^{-6}\text{M}$  SPD ( $31.3 \pm 3.1 \mu\text{mol O}_2 \text{ evolved h}^{-1} \text{g}^{-1}\text{FW}$  in 18psu+SPD as compared to  $16.7 \pm 0.5 \mu\text{mol O}_2 \text{ evolved h}^{-1} \text{g}^{-1}\text{FW}$  in the control 18psu without SPD).

Regarding enzymatic activities (Fig. 2), the hyposaline treatment caused a significant decrease in ODC activity, whereas ADC and arginase remained constant. On the contrary, the results provided evidence that a significant decrease in TGase is promoted by hyposaline conditions (five-fold reduction, as the highest modification observed). TGases do exist in plants and microalgae, but, to our knowledge, this is the first report on TGase activity as related to hyposaline stress in a marine macroalga. These variations between free and bound PAs would explain how total PAs were not significantly different between treatments ( $26.14 \pm 4.5 \mu\text{g g}^{-1}\text{FW}$  in samples at 18psu and  $19.76 \pm 4.2 \mu\text{g g}^{-1}\text{FW}$  at 36psu).

In conclusion, a moderate hyposaline shock caused an increase in the free fraction of the common PAs, PUT, SPD and SPM, mainly due to a decrease in TGase activity. This simple mechanism may account for the benefits in physiological performance during acclimation, since the photosynthetic rate increased in thalli when exposed to free PAs.



**Figure 2.** ADC, ODC, TGase and arginase ( $\times 10^{-1}$ ) enzymatic activities (mmole product  $\text{mg}^{-1}\text{protein h}^{-1}$ ) after 24h in seawater at 36 and 18psu. The data are the mean of three samples  $\pm$  standard error. The same letter denotes significant differences between treatments ( $P < 0.05$ ).

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