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SHORT REPORTS

IDENTIFICATION OF ABSCISIC ACID IN THE SEAWEED *ASCOPHYLLUM NODOSUM*

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Key Word Index—*Ascophyllum nodosum*, Phaeophyta, seaweed, algae, abscisic acid, phytohormone, growth regulator.

Abstract—Abscisic acid (ABA) has been isolated from commercial preparations and freeze-dried samples of the brown algae *Ascophyllum nodosum*. Purification by established methods yielded 0.03–0.15 μg ABA/g dry weight in lyophilized powders and 0.10–0.46 μg ABA/g dry wt in a commercial extract. These values are similar to those reported for higher plants. The presence of abscisic acid was confirmed by mass spectroscopy, providing an unambiguous identification of ABA in extracts of macroalgae.

INTRODUCTION

Ascophyllum nodosum (L.) Le Jol. is a brown intertidal alga currently harvested for use as a fertilizer supplement. This use of seaweeds as supplements is of interest since their effects on higher plants often cannot be explained in terms of macronutrient content (N, P, K) alone [1]. Seaweeds contain substances with plant hormone-like activity. Compounds that co-chromatograph with abscisic acid (ABA, **1**), a higher plant growth inhibitor, have been reported in seaweed extracts [2, 3] but other workers report that ABA does not occur in algae [4, 5]. Chemical evidence supporting this presence or absence of ABA is lacking. Kingman and Moore [6] reported a high level of abscisic acid in a commercial extract of *Ascophyllum nodosum* as determined by GC-FID. They did not, however, confirm the identity of the compound by GC-MS, an important omission because Niemann and Dorffling [5] have reported the presence of a compound in *Enteromorpha compressa* that co-migrates on GLC with methyl ABA. Recently Tietz and Kasprik [7] examined three genera of green algae for the occurrence of abscisic acid. *Fritschiella tuberosa* and *Draparnaldia plumosa* did not contain ABA, but ABA was identified by GC-MS in cultures of *Stigeoclonium* sp. grown in synthetic medium. We report here the occurrence and concentrations of abscisic acid in a lyophilized sample and in a commercial extract of the brown seaweed *Ascophyllum nodosum* This is the first mass spectrometric identification of ABA in a marine macroalgae.

RESULTS AND DISCUSSION

The level of ABA in lyophilized powders of *Ascophyllum* and a commercial *Ascophyllum* preparation (Nitrozyme ™) was determined Each sample was divided into equal aliquots. One aliquot was spiked with [^{3}H]ABA and ABA (as its methy ester) was measured in the spiked and unspiked samples after purification by HPLC by capillary GC-ECD (Table 1). Average recoveries, as determined by the recovery of added [^{3}H]ABA, were 32% for the commercial extracts and 29% for the lyophilized samples. Unspiked aliquots from both the lyophilized powder and the commercial extract were analysed by GC-MS to confirm the identity of ABA. The resulting mass spectra were identical to those of authentic ABA methyl ester and published spectra [8]. A small amount of 2-*trans*-methyl ABA was also present in the purified samples. Its level could be increased by exposure to light and was due to the photoisomerization of 2-*cis* ABA after purification.

The range 0.03–0.15 μg ABA/g dry wt in lyophilized powders of *Ascophyllum* is similar to values for ABA observed in higher plants and cultures of the green algae *Stigeoclonium* sp (Table 1). A previous report [6] on abscisic acid in a commercial extract of *Ascophyllum* suggested it may be extremely rich in ABA. One possible explanation is that ABA may be released from a bound form, such as ABA glucose ester [9], by base hydrolysis used during commercial processing. However, in this study, the ABA content in a commercial preparation of *Ascophyllum* from an area close to where we obtained our material had a similar ABA content to that observed in the freshly collected algae (Table 1). The concentration of ABA was not increased in the lyophilized powders by base hydrolysis (data not shown). Thus we conclude that

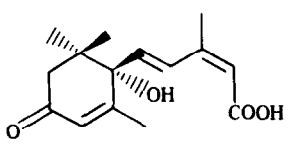

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Table 1 Amounts of abscisic acid in *Ascophyllum nodosum* preparations and representative higher plants and algae

Species	μg/g dry wt*	Reference
Ascophyllum nodosum		
Commercial extract ($n = 5$)	0 10–0 46†	[This study]
Lyophilized seaweed ($n = 3$)	0 03–0 15	[This study]
Higher plants (non water-stressed leaves)		
Spinacia oleracea	0 13–0 21	[9]
Phaseolus vulgaris	0 30–0 60	[12]
Xanthium strumarium	1 70–3 50	[10]
Algae		
Stigeoclonium sp	0.014–0 044	[7]
Ascophyllum (commercial extract)	20 000	[6]

*For comparison purposes, 1 g dry wt = 10 g fr wt
†0 03–0 14 μg per ml extract

the ABA content in freshly prepared *Ascophyllum* extracts and commercially available preparations are similar, and that these values are in line with what has been generally observed for turgid leaves in higher plants Abscisic acid was unambiguously identified by GC-MS in the extracts of *Ascophyllum* However, we do not claim that it is necessarily produced by the algae These algal preparation were not axenic and *Ascophyllum nodosum* is known to harbour several epiphytic species The function of ABA in these algae is also unknown ABA in higher plants is involved in the control of stomatal opening during times of water stress [10, 11]. *Ascophyllum* occupies the intertidal zone and is subject to extreme periods of desiccation However its thalli do not contain stomata as observed in higher plants The role of plant growth inhibitors in the growth of macroalgae is a subject of continuing investigation

EXPERIMENTAL

Ascophyllum nodosum was collected from the mid-intertidal zone behind Bigelow Laboratory for Ocean Sciences (West Boothbay Harbour, Maine, USA 04575). Epiphytic algae were removed by hand, the algae pulverized in liquid N_2, lyophilized, and stored at −20°. Freeze-dried samples (100 g) were homogenized in 80% aqueous Me_2CO containing 1% HOAc and 0 01% 2,6-di-*t*-butyl-4-methylphenol as an antioxidant The Me_2CO extract was divided into two equal aliquots and racemic [^{3}H]ABA (50 000 cpm, 42 2 mCi/mmol) added to one aliquot For analysis of the commercial seaweed extracts (Nitrozyme™ containing *ca* 30% dissolved solids, Atlantic Laboratories, Waldoboro, ME 04572), duplicate 20–50 ml aliquots were diluted with an equal volume of H_2O After spiking one aliquot with [^{3}H]ABA, the extracts were partitioned into acidified EtOAc and back extracted into 0 2 M $NaHCO_3$ Following a second extraction into acidified EtOAc, ABA was purified by HPLC using a modification of published methods [10] The initial step involved chromatography on a Partisil ODS-3 column (Whatman, 10 μ, 4 6 × 250 mm, 2 ml/min) using a linear gradient (5%/min) of 30 to 100% EtOH in 1% HOAc Fractions containing abscisic acid (10–12 min) were pooled, lyophilized, and methylated with CH_2N_2 Methyl ABA was chromatographed using the same column (30–100% MeOH in H_2O, 2 ml/min, 5%/min) and the appropriate fractions (18–22 min) pooled A final step consisted of normal phase HPLC (Dupont Zorbax-sil, 10 μ, 4 6 × 250 mm, 2 ml/min) using isocratic 6% *i*-PrOH in hexane as solvent Fractions containing MeABA (8–10 min) were pooled, taken to dryness under N_2, and quantified by capillary GC-ECD (60 m SPB-1 column, 150–250 at 12 5°/min, 1 ml/min N_2, 2 μl direct injection) Spiked samples were corrected for the amount of added ABA remaining after purification by subtraction using the specific radioactivity (42 2 mCi/mmol) of the spike Identification of ABA in the unspiked samples was confirmed by GC-MS using a 6 m SPB-1 column (50–300° at 5°/min, 70 eV), *m/z* (rel int) 260 [M $-H_2O]^+$ (2), 246 [M − HOMe]$^+$ (2), 222 [M − 56]$^+$ (1), 205 (3), 190 [222 − MeOH]$^+$ (100), 162 [190 − CO]$^+$ (34), 147 (15), 134 [162 − CO]$^+$ (40), 125 [sidechain]$^+$ (40), 119 (10), 112 (8), 105 (11), 91 (29), 77 (14). 2-*trans*-Methyl ABA was identified by comparison of its R_f and mass spectrum with a known standard

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