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LOW MW CARBOHYDRATES AND IONS IN RHODOPHYCEAE: QUANTITATIVE MEASUREMENT OF FLORIDOSIDE AND DIGENEASIDE

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Abstract—The content of the heterosides floridoside and digeneaside and of the main ions Na^+, K^+, and Cl^- was estimated in 20 species of the Rhodophyceae. Methods for quantitative determination of the heterosides are described. The floridoside content is in the range of 1.5–8% on a dry weight basis (*Catenella*: up to 22%); the content of digeneaside, exclusively found in species of the Ceramiales, is lower, in the range of 1–2.2% on a dry weight basis. All species investigated have Cl^- as main anion, while there is a remarkable diversity in cation composition. Na^+ was the major cation in 12 of the species investigated, the others having K^+ as main cation.

INTRODUCTION

Carbohydrates in marine algae have been intensively investigated with respect to polysaccharides [1–3]. Different algal classes have characteristic carbohydrate compositions which is therefore of taxonomic interest [4]. In Rhodophyceae two groups can be distinguished by the difference between their major low MW photosynthetic products:

One group produces digeneaside (2-D-glycerate-α-D-mannopyranoside; [5]). This is restricted to species of the order Ceramiales [6, 7]. The second group including all other orders of the Rhodophyceae contains floridoside (2-*O*-D-glycerol-α-D-galactopyranoside; [8]) [6, 7, 9, 10]. Both compounds are rapidly labelled during $^{14}CO_2$-fixation [7, 11].

In this paper methods are described for a quantitative estimation of floridoside and digeneaside. These compounds play a role in osmotic adaptation probably as an important osmoticum in the cytoplasm [11, 12]. Hence information on the concentration of low MW organic solutes is necessary. In addition, the contents of the main ions Na^+, K^+, and Cl^- are determined. It was thought that marine algae generally have a high K^+ concentration and a low Na^+ level, similar to the ion composition found in many higher plants [13]. However, several exceptions are known and the K^+/Na^+ ratio is very variable in different species [11, 14–16]. Therefore, a more systematic investigation of ion composition of species belonging to the same class is desirable.

RESULTS AND DISCUSSION

The methods of quantitative estimation of floridoside and digeneaside are based on a hydrolytic cleavage of the heterosides and an enzymatic determination of the resulting glycerol (deriving from floridoside) and glyceric acid (from digeneaside). This has the advantage of high sensitivity and selectivity, so that crude extracts can be used. Estimation of the sugar moiety of the heterosides leads to overestimations because of inevitable contamination of the crude extracts with sugars deriving from oligo- and polysaccharides after hydrolysis.

Two methods of hydrolysis were tested. Extracts containing floridoside were incubated with α-galactosidase, a method which was successfully used for cleavage of iso-floridoside in extracts of *Ochromonas*, a Chrysophyceaen alga [17]. Extracts with digeneaside were treated with α-mannosidase. In a second series the extracts were hydrolysed with 1 M HCl. For both substances enzymatic cleavage was incomplete. Hence for further investigation, the acid hydrolysis was chosen, which is in addition more economic and more rapid. Recovery of glycerol artificially added to acid hydrolysed extracts was better than 97% with the enzymatic assay used. Similar results were obtained with glyceric acid estimation using a method which has been described for higher plants [18] in which the glyceric acid is determined indirectly from the NADH produced by enzymatic reduction of NAD. As the yield of NADH is only 70–80% of the theoretical yield [18], the pH of the reaction was varied from the value of 10.2 described, but only lower yields were obtained.

Contents of low MW carbohydrates

The total amount of floridoside varies with species (Table 1). *Catenella nipea* was the species with the highest concentration on a fr. wt basis, equivalent to *ca* 22% of the dry wt. The species with the lowest content was *Lomentaria umbellata* which accumulates *ca* 2% floridoside on a dry wt basis. In *Corallina*

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Table 1. Ion and floridoside content in Raphodophyceae

Taxon	Ions (μmol/g fr. wt) Na^+	K^+	Cl^-	Floridoside (μmol/g fr. wt)	Floridoside (% of dry wt)	Dry wt (% of fr. wt)
Nemalionales						
Delisea pulchra (Grev.) Mont.	380±22	112±29	405±31	11.8±0.7	1.6	19±9.6
Gelidiales						
Gelidium australe J. Ag.	314±48	144±27	411±29	15.6±1.4	2.5	16±1.1
Gelidium caulacantheum J. Ag.	217±14	124±34	340±18	17.6±2.6	2.4	19±0.5
Gigartinales						
Catenella nipae Zan.	282±23	30±0.6	305±38	133±9	22.6	15±0.2
Gracilaria edulis (Gmel.) Silva	84±10	396±19	317±18	11.2±4.2	2.0	14±0.2
Gracilaria verrucosa (Hudson) Papenfuss	143±16	323±12	362±41	46±2	7.9	15±0.7
Hypnea valentiae (Turn.) Mont.	369±30	72±16	350±39	12±4.4	1.9	16±0.2
Rhabdonia nigrescens Harv.	258±12	203±38	493±51	6.1±0.7	1.1	14±0.1
Cryptonemiales						
Corallina officinalis L.	70±4.5	116±11	101±12	14.5±2.2	0.6	65±5.5
Grateloupia australis J. Ag.	278±36	33±7.2	270±21	27.1±6.6	3.3	21±0.3
Grateloupia filicina (Lamour.) C. Ag.	456±68	63±8.4	369±103	10.4±2.1	1.7	16±0.3
Rhodymeniales						
Rhodymenia foliifera Harv.	237±25	130±21	271±41	46.7±11.9	4.8	25±0.7
Lomentaria umbellata (H.u.H.) Yendo	326±6	21±2.6	330±34	8.8±0.6	1.6	14±0.15

The data are means ± standard deviation (4–9 replicates). The ion contents are corrected for seawater contamination (free space). Dry wt is given as per cent of fr. wt (5 replicates) to allow recalculation for comparison with data in the literature.

officinalis the floridoside content based on weight is misleading due to the high degree of calcification (dry wt 65% of fr. wt, Table 1).

Calculating the floridoside content of *Corallina* on a cell water basis and correcting for the free space ('inulin-[^{14}C] space') resulted in concentrations of *ca* 65 mM [11]. For comparison, the concentrations of floridoside are in *Catenella* 174 mM, in *Rhodymenia* 60 mM and in *Lomentaria* 12 mM. Species-dependent variation in the content of floridoside was also reported from *Porphyra umbilicalis* (8.4% on a dry wt basis) and *Chondrus crispus* (*ca* 5.9% of dry wt) [19]. By GLC a floridoside content of 2.6% of dry wt was found in *Porphyra umbelicalis* and 1.5% in *Furcellaria fastigiata* [20].

Digeneaside, the major low MW carbohydrate accumulated in species of the order Ceramiales (except the genus *Bostrychia* [21]), did not exceed 2.2% of dry wt in *Centroceras*, the species with the highest content measured (Table 2). In most species investigated it was less than 1% of dry wt.

A lower heteroside content was found in samples collected at Spring (October). The values for floridoside are (in μmol per g fr. wt, 3 measurements): *Hypnea* 6.3±0.8; *Grateloupia filicina* 8.9±1; *Rhodymenia* 38.6±1.5. In species containing digeneaside the content was (in μmol per g fr. wt, 3 measurements): *Griffithsia* 2.8±1 and *Callithamnion* 4.6±0.5. The effect of salinity on the content of organic solutes in marine algae was investigated in detail by Kirst and Bisson [11].

Ion composition

In 12 out of 20 species tested, Na^+ is the main cation (Tables 1 and 2). Na^+-rich species in marine algae seem to be much more frequent, as originally concluded (cf. [13]). There is no correlation between taxonomic groups and ion composition. This is consistent with findings in other algal classes: Chlorophyceae and Phaeophyceae [11, 22, 23]. Species collected from the same place have a very different ion composition, hence environmental factors cannot explain the diversity.

It is known that in K^+-free artificial seawater *Porphyra perforta* loses K^+ and accumulates Na^+ instead [24]. When subjected to high hyper-osmotic conditions by increasing the NaCl concentration in the medium, many marine algae decrease K^+ concentration, concomitantly increasing the Na^+ concentration, if K^+ was the major cation [11, 25]. Gutknecht [26] has shown that *Gracilaria foliifera* maintained its high intracellular K^+/Na^+ ratio, but that *Ulva lactuca* and *Fucus vesiculosus* were unable to maintain their normal high K^+/Na^+ ratio under anaerobic conditions, losing over 50% of internal K^+ within 24 hr. Hence, K^+/Na^+ ratios vary, depending on conditions.

Table 2. Ion and digeneaside content in species of the Ceramiales (Rhodophyceae)

Taxon	Ions (μmol/g fr. wt) Na^+	K^+	Cl^-	Digeneaside (μ mol/g fr. wt)	(% of dry wt)	Dry wt (% of fr. wt)
Ceramiales						
Anotrichium tenue (Nägeli) (syn. *Griffithsia tennis*)	179±12	261±51	433±39	7.4±1.4	1.0	20±0.8
Callithamnion spec.	198±35	217±13	358±65	8.3±4.6	0.9	25±1
Chondria dasyphylla (Woodw.) C. Ag.	261±14	136±38	456±27	2.6±0.9	0.7	11±0.3
Centroceras clavulatum (Ag.) Montagne	90±11	297±28	285±25	15.8±4.6	2.2	20±0.3
Griffithsia monilis Harvey	78±16	510±67	437±26	3.2±0.5	0.9	10±0.2
Laurencia obtusa (Huds.) Lamour	383±50	30±3	358±25	3.5±0.4	0.8	12±0.2
Spyridia filamentosa Harv. (syn. *Spyridia biannulata*)	121±21	128±36	380±68	6.1±0.5	0.8	20±0.6

For further details see Table 1.

However, the factors which cause the different cation composition under the steady state reported here are unknown. It has to be emphasized that in spite of high overall Na^+ concentrations, K^+ is very likely the main cation in the cytoplasm [15, 16].

The Cl^- content does not balance the charge on K^+ and Na^+ exactly (Tables 1 and 2). A remarkable difference between Cl^- content of species which belong to the Ceramialias containing the negatively-charged digeneaside and the other red algae could not be confirmed because of the large scatter in Cl^- content and the relatively low concentration of digeneaside.

Cl^- content was highest in the large vacuolated 'giant cells' of *Griffithsia*, in agreement with the generally observed accumulation of this anion in the vacuole [15]. In smaller cytoplasm-rich cells, Cl^- concentration was lower. An example of an extremely low Cl^- concentration (81 mM Cl^-; 482 mM K^+; 51 mM Na^+) is the vacuole-less *Porphyra perforata* [27]. In *Corallina officinalis* the ion content cannot be based on fr. wt (see above). Calculations on a cell water basis yielded 418 mM Cl^-, 291 mM Na^+, and 525 mM K^+ [11].

The accumulation of the heterosides digeneaside in Cermiales and floridoside in the other orders of the Rhodophyceae is in good agreement with the taxonomy. However, the diversity in ion composition even within closely related species from the same habitat manifests remarkable differences in transport physiology.

EXPERIMENTAL

Algae listed in Tables 1 and 2 were collected during Summer (December 1978) in Port Jackson and Botany Bay, Sydney, with the exception of *Laurencia obtusa* which originated from Heron Island (Capricorn group, Great Barrier Reef). All specimens were carefully cleaned, blotted, weighed and killed in 100% EtOH immediately after collection. Parts of the fresh thalli were taken for estimation of dry wt (24 hr at 105°).

Extraction and ion determination. Tissues (0.5–1 g fr. wt) were minced, then extracted with 3 ml 70% EtOH, 6–9 times, and heated to 75° for 30 min at each step. Combined extracts were used for ion analysis: Na^+ and K^+ were determined by atomic absorption spectrophotometry and Cl^- by electrometric titration. Ion contents were corrected for seawater contamination by estimating the free space with inulin-[^{14}C] as described in ref. [22].

Hydrolysis. Aliquots (8–12 ml) of the extracts were evapd to dryness in a vacuum desiccator at room temp. Residues were redissolved in 1 ml 25% EtOH. For enzymatic digestion, 0.2 ml of this extract was mixed with 0.2 ml NaOAc buffer (0.1 M, pH 5.6) and incubated (25°, 48 hr) with 0.01 ml α-galactosidase (EC 3.2.1.22; enzyme suspension (Boehringer No. 105 023); final content in the test was 0.05 units. Following ref. [17], the soln was covered with toluene during incubation to prevent bacterial contamination. Extracts from species belonging to the Ceramiales (Table 2) were subjected to the same procedure except that α-mannosidase was used (EC 3.2.1.24, Boehringer No. 107 379; 0.05 units per test). Acid hydrolysis was performed with 0.2 ml algal extract and 0.2 ml 2 M HCl in sealed ampoules, incubated at 95° for 6–12 hr. After neutralization with 10 M NaOH the soln was centrifuged and the clear supernatant used for glycerol or glyceric acid analysis. Glycerol was determined spectrophotometrically with Boehringer test combination (No. 125 032). In this test glycerol is phosphorylated to glycerol-3-phosphate with ATP and glycerokinase. The resulting ADP is used in pyruvate synthesis deriving from PEP with pyruvate kinase. Finally pyruvate and NADH together with lactate dehydrogenase produces lactate. The decrease in NADH is proportional to the concn of glycerol. Glyceric acid was estimated according to the method described in ref. [18]. The method is based on the oxidation of glyceric acid to hydroxy pyruvic acid by NAD^+ and glyceric acid dehydrogenase. Hydroxypyruvic acid formed is destroyed by H_2O_2. The assay contained in a total vol. of 2.1 ml: 0.1 M 2-amino-2-methylpropanol and 2-amino-2-methylpropanolhydrochloride as buffer (pH 10.2), 1 mM EDTA, fresh prepared solns of H_2O_2 (4 mM final concn) and of NAD (1 mM final concn) with dithiothreitol (1.3 mM final concn) added. Either 0.1 ml sample of glyceric acid standard (20–80 nmol) or a H_2O reagent blank was added. The reaction was started with 0.01 ml (2.8 units) glyceric acid dehydrogenase/glyoxylate reductase (EC 1.1.1.26; Sigma No. G-3504). The NADH produced was measured with a fluorescence spectrophotometer. After 30–40 min the reaction was complete, but with high glyceric acid contents the incubation time was extended to 60–80 min. The glyceric acid concn was

calculated after correcting for the reagent blank and by using standards giving readings in the same range of emission. Samples from non-hydrolysed extracts were also tested to correct for free glycerate and glycollate.

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