

Chapter 18

Brown Algal Phlorotannins: An Overview of Their Functional Roles



Iván Gómez and Pirjo Huovinen

Abstract Phlorotannins are polyphenolic compounds, relatively hydrophilic, formed by polymers of phloroglucinol and found exclusively in brown algae. These molecules are located in vesicles denominated physodes (the soluble fraction) and also complexed to polysaccharides in the cell wall (the insoluble fraction). Well known as potential grazer deterrents, one of the most striking characteristics of these compounds, due to a number of hydroxyl groups, is their antioxidant potential, which opens promising perspectives for pharmaceutical and biotechnological uses. In Antarctic brown algae, especially endemic species of Desmarestiales, constitutively high levels of phlorotannins (up to 12% of dry weight) have been measured. Although translocation has not been conclusively confirmed, the differential allocation of phlorotannins in meristematic and reproductive tissues in some species suggests their involvement in chemical defenses protecting essential metabolic functions. Due to their UV-absorbing properties and peripheral localization in cells and tissues, phlorotannins have been related with the increased tolerance to UV radiation in various Antarctic brown algae. However, no induction of phlorotannins by UV has been demonstrated, which strongly supports the idea that these molecules are constitutive biochemical components of a suite of mechanisms against multiple stressors. Due to their structural role as primary compounds, phlorotannins are essential for various morpho-functional processes that in the case of Antarctic algae allow them to thrive under extreme conditions. Overall, the significance of phlorotannins in this group of algae has largely been recognized; however, fundamental aspects of their molecular expression, synthesis, and regulation still need to be addressed, especially considering the climate change-driven environmental scenarios.

Keywords Antioxidant activity · Brown algae · Phenolic compounds · Physodes · Secondary metabolites · UV-absorbing compounds

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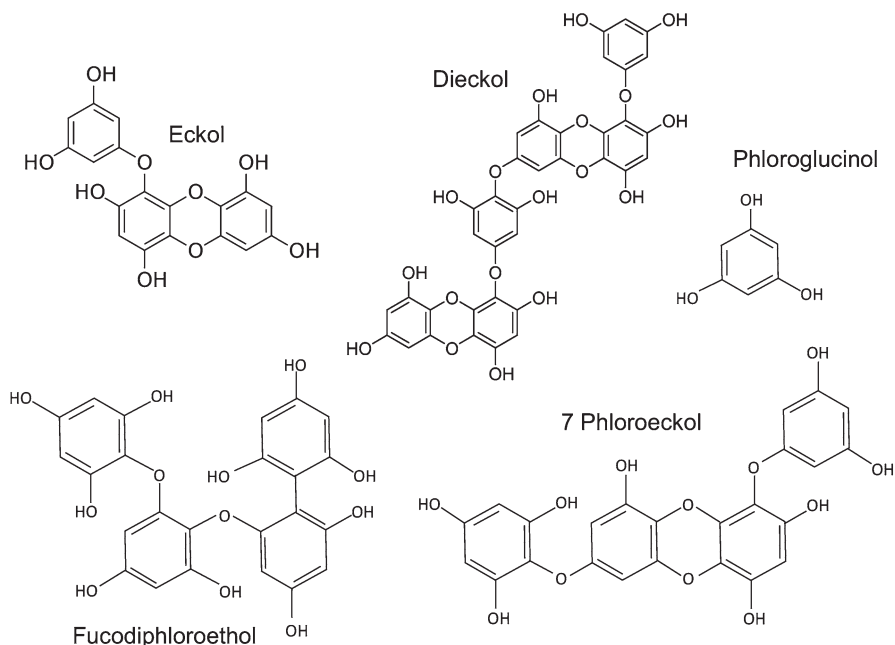


Fig. 18.1 Chemical structure of some common phlorotannins extracted from brown algae

18.1 Introduction

Brown algal phenolics belong to a single structural class, the phlorotannins, which are dehydropolymers of phloroglucinol (1,3,5-trihydroxybenzen). Depending on the degree of polymerization, phlorotannins present a wide range of molecular weights (between 140 and 40.000 Da; Ragan and Glombitza 1986). Thus, based on the number of phloroglucinol units, the profiling of phlorotannins can vary considerably among species (Steevensz et al. 2012). Within the phlorotannins found in brown algae, the most common are fucols, phlorethols, fucophlorethols, and eckols (Fig. 18.1), which vary depending on the type of chemical linkage, e.g., ether Aryl-O-Aryl linkages in phlorethols or dibenzodioxin linkages in eckols (Ragan and Glombitza 1986; La Barre et al. 2010). As secondary metabolites, these substances play a series of putative roles in the cell, mainly as anti-herbivory defense, antifouling activity, UV protectants, and antioxidants. Phlorotannins can be present as soluble substances sequestered in vesicle-denominated physodes and as insoluble fraction bound to polysaccharides in the cell wall (denominated the insoluble fraction) (Fig. 18.2). Due to this, these compounds are regarded also as primary compounds, essential during cell formation (Schoenwaelder 2002). This has been corroborated by histological studies indicating that phlorotannins

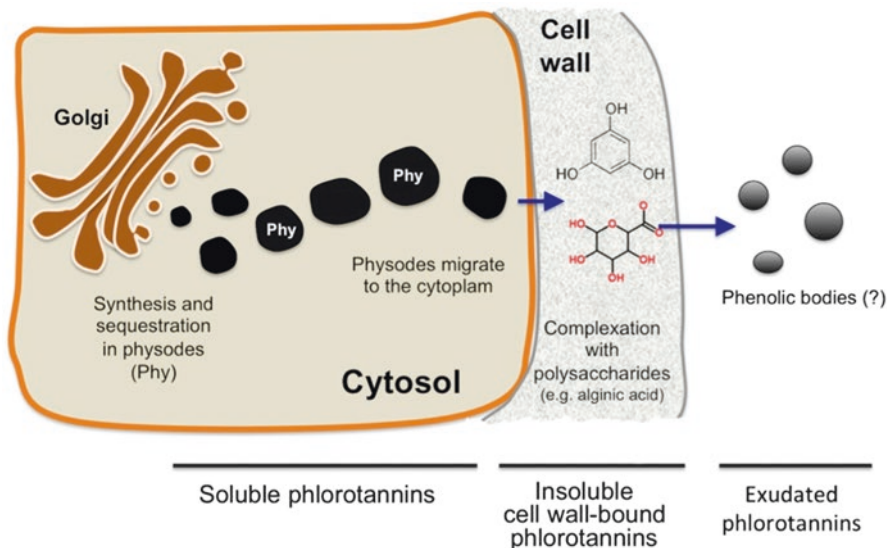


Fig. 18.2 Localization of physodes and the different functional forms of phlorotannins in brown algal cells. The relationship between exuded phlorotannins and extracellular phenolic bodies has not been conclusively established

participate actively in wall configuration of zygotes (Kevekordes and Clayton 1999), are concentrated at the periphery of multicellular embryos of *Fucus* (Schoenwaelder and Clayton 1998), and are actively synthesized during wound healing and sealing after amputation of thallus regions simulating herbivory (Lüder and Clayton 2004).

Because of their ubiquity, phlorotannins can be present in high concentrations in some species. For example, in Antarctic brown algae, particularly endemic species of Desmarestiales, constitutively high levels of phlorotannins have been measured, forming up to 12% of dry weight (Table 18.1). However, it has been well demonstrated that total phlorotannin content can vary significantly depending on a number of biotic and abiotic factors (Van Alstyne and Pelletreau 2000; Pavia and Toth 2000; Jormalainen et al. 2003; Gómez and Huovinen 2010).

Apart from their well-known role as herbivore deterrents (reviewed in Chap. 17 in this volume and by Iken et al. 2009), phlorotannins and various of their chemical derivatives apparently have other important functions, especially in oxidative metabolism and metal chelation (Stauber and Florence 1987), with far-reaching implications for ecophysiology of brown algae (La Barre et al. 2010).

Table 18.1 Concentration of total phlorotannins (soluble and insoluble) in different Antarctic brown algae

Species	Phlorotannin contents		Remarks	Reference
	Soluble	Insoluble		
	Percentage (mg g ⁻¹ DW)			
<i>Desmarestia anceps</i>	117 ± 3		Average of different thallus parts (Anvers Island)	Fairhead et al. (2005a)
	60 ± 19		1 and 37 m (Anvers Island)	Iken et al. (2007)
	60 ± 5.5		28 m (King George Island)	Huovinen and Gómez (2013)
	30		10–15 m (Anvers Island)	Schoenrock et al. (2015)
	80 ± 18	28 ± 4.9	20 m (King George Island)	Gómez and Huovinen (2015)
	52 ± 15		15 m (King George Island)	Flores-Molina et al. (2016)
	43–48	33	7–10 m depth (King George Island)	Rautenberger et al. (2015)
<i>Desmarestia menziesii</i>	52 ± 2		Average of different thallus parts (Anvers Island)	Fairhead et al. (2005a)
	30 ± 12		1 and 37 m (Anvers Island)	Iken et al. (2007)
	85 ± 5.6		Apical parts; 17 m (King George Island)	Huovinen and Gómez (2013)
	10		10–15 m (Anvers Island)	Schoenrock et al. (2015)
	55 ± 15		20 m (King George Island)	Gómez and Huovinen (2015)
	55–58	18	7–10 m depth (King George Island)	Rautenberger et al. (2015)
<i>Ascoseira mirabilis</i>	35 ± 25		1 and 37 m (Anvers Island)	Iken et al. (2007)
	15 ± 12		8 m (King George Island)	Huovinen and Gómez (2013)
	12 ± 3	50 ± 10	1 m (King George Island)	Huovinen and Gómez (2015)
	13 ± 3	15 ± 2.4	20 m (King George Island)	Gómez and Huovinen (2015)
	10–12	20–23	7–10 m depth (King George Island)	Rautenberger et al. (2015)
<i>Himantothallus grandifolius</i>	63 ± 8		1 and 37 m (Anvers Island)	Iken et al. (2007)
	120 ± 8.6		30 m (King George Island)	Huovinen and Gómez (2013)
	97 ± 20	49 ± 11	20 m (King George Island)	Gómez and Huovinen (2015)
	90–99	40	7–10 m depth (King George Island)	Rautenberger et al. (2015)

(continued)

Table 18.1 (continued)

Species	Phlorotannin contents		Remarks	Reference
	Soluble	Insoluble		
<i>Cystosphaera jacquinotii</i>	26 ± 16		1 and 37 m (Anvers Island)	Iken et al. (2007)
	29 ± 5.2		25 m (King George Island)	Huovinen and Gómez (2013)
	75 ± 19	10 ± 3	15–20 m (King George Island)	Huovinen and Gómez (2015)
<i>Desmarestia antarctica</i>	42 ± 5.7		20 m (King George Island)	Huovinen and Gómez (2013)
<i>Adenocystis utricularis</i>	52 ± 4.4		Intertidal zone (King George Island)	Huovinen and Gómez (2013)

18.2 Synthesis and Cellular Localization of Phlorotannins: Dual Functions as Secondary Metabolites and Structural Compounds

Phlorotannins are synthesized via the acetate-malonate pathway through a type III polyketide synthase (Herbert 1989; Meslet-Cladiere et al. 2013), and in terms of their chemical properties, they differ from the condensed tannins of vascular plants (Arnold and Targett 2002). It has been suggested that some fatty acids associated with the Acetyl-CoA, a key intermediate in the polyketide pathway, could be regarded as precursors of phlorotannins (Steinhoff et al. 2011). However, metabolism of phenolics in algae has been much less studied, and key aspects of biosynthesis and regulation are unknown.

Due to their reactivity, phlorotannins may easily form complexes with macromolecules, and they are sequestered in physodes through the formation of covalent, hydrogen, or ionic bonds (the soluble fraction) (Fig. 18.2). Traditionally physodes or phenolic precursors are thought to be synthesized in the chloroplast or at the chloroplast membrane, and various authors have described an osmiophilic material being released from chloroplasts (Evans and Holligan 1972; Feldmann and Guglielmi 1972; Pellegrini 1980). An alternative explanation suggests that phenolic material is produced in the chloroplast endoplasmic reticulum (CER), which may play a role in the transport of phenolic precursors to cell vacuoles and physodes (Pellegrini 1980; Clayton and Beakes 1983; Kaur and Vijayaraghavan 1992) or may directly give rise to physodes (Feldmann and Guglielmi 1972; Oliveira and Bisalputra 1973). When the physodes make contact with the plasmalemma, phlorotannins are released and polymerized in the apoplast (cell wall) forming complexes with polysaccharides, e.g., alginic-acid-bound phlorotannins (Schoenwaelder and Clayton 1999) (Fig. 18.2). Some studies have identified peroxidases in the cell wall of *Ascophyllum nodosum* suggesting that phlorotannins excreted from the cells may be modified through the activity of these enzymes (Vilter 1995). In fact, the process

of oxidative condensation and the linkage to alginic acids in the apoplast is apparently driven by vanadium-dependent haloperoxidases (Potin and Leblanc 2006; Salgado et al. 2009). Vreeland and Laetsch (1988) proposed that phenolic cross-linking of alginate may occur in early wall formation in *Fucus* and that peroxidases may be involved in the catalysis of phenolic condensation into alginate. During early phases of growth, physode movements to regions of active wall formation from the cell periphery to the rhizoid tip and to the impending plane of cytokinesis are dependent on interactions with the cytoskeleton (Schoenwaelder and Clayton 1999). Hence, actin microfilaments may be acting as a general circulatory system moving physodes around the cell, with microtubules directing physodes (and probably other wall components) to cell-wall deposition sites, both in the primary wall and at cross-walls (Kevekordes and Clayton 1999; Schoenwaelder and Clayton 1999). Although the exudation of phlorotannins has been reported (Ragan and Glombitza 1986; Toth and Pavia 2002; Koivikko et al. 2005), no clear evidence exists that they are related with phenolic bodies described in embryos of *Durvillaea antarctica* (Kevekordes and Clayton 1999). For example, it was demonstrated that extracellular excretion of phenolic compounds in *Eisenia bicyclis* and *Ecklonia kurome* corresponded to monomeric bromophenols, while phloroglucinol or polymeric phlorotannins were not detected (Shibata et al. 2006). On the other hand, phlorotannins are strong chelators of metals, and thus, they are thought to participate in exudation-based detoxification mechanisms: they may sequester metal ions in physodes (Smith and Harwood 1986), and through exudation processes, these metal-complexing compounds may decrease the metal concentration or alter its speciation in the surrounding water (Gledhill et al. 1999). As physodes are more abundant in peripheral cell layers, their role as a filter stopping metals from entering the inner cells has been proposed (reviewed by Schoenwaelder 2002).

18.3 Phlorotannins as UV-Screening Substances

In contrast to terrestrial plants, where natural levels of UV radiation do not necessarily result in damage (Paul and Gwynn-Jones 2003; Hideg et al. 2013), aquatic organisms, especially subtidal seaweeds, can be impaired when they are exposed to high solar radiation (Bischof et al. 2006a). Thus, synthesis and accumulation of UV-screening substances is a common photoprotective strategy observed in several groups of living organisms (Karentz et al. 1991; García-Pichel and Castenholz 1993; Cockell and Knowland 1999). In fact, various of these compounds have been isolated and tested as bioactive substances for use in skin care, cosmetics, and pharmaceutical products (reviewed in Pangestuti et al. 2018). Due to their chemical properties, diverse phenolics are regarded as general anti-stress agents, including UV protection and antioxidant activity, and apart from brown algae, they have been reported in green (Menzel et al. 1983; Pérez-Rodríguez et al. 1998, 2001; Gómez et al. 1998; Ross et al. 2005) and red algae (Athukorala et al. 2003; Yildiz et al. 2011; Heffernan et al. 2014; Cruces et al. 2018).

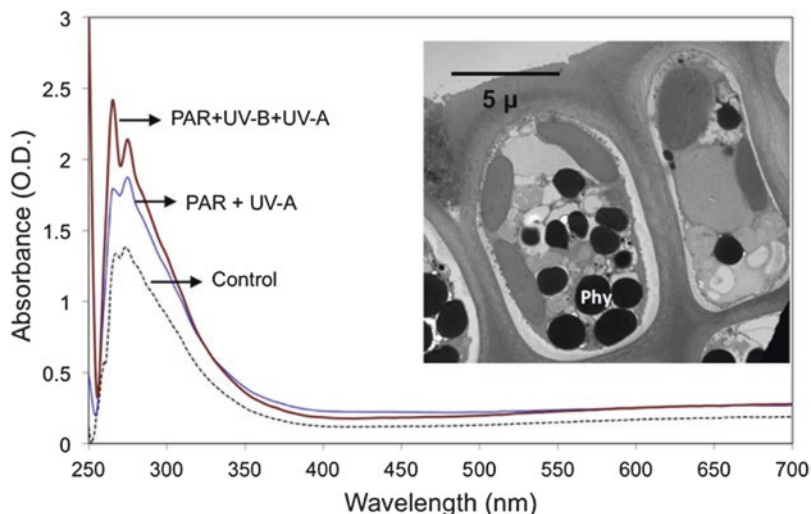


Fig. 18.3 Absorption of ethanol-water extract of apical fronds of *Lessonia spicata* exposed to UV radiation for 24 h

Phlorotannins have been correlated with an increased tolerance to UV radiation (Pavia et al. 1997; Swanson and Fox 2007; Gómez and Huovinen 2010; Steinhoff 2010). In general, phlorotannins absorb at wavelengths between 200 and 300 nm, i.e., well in the UV-C range and at shorter UV-B wavelengths. When phlorotannins are removed using polyvinylpyrrolidone (PVPP), absorption of algal extract decreases by 70% in the range between 280 and 300 nm (Pavia et al. 1997). Although the effectiveness of phlorotannins as UV-screening compounds is higher at the UV-C range, absorption spectra of the different fractions of phlorotannins (phy-sodes, cell-wall-bound and excreted phlorotannins) may be different. In fact, the absorption peak of the trihydroxy-coumarin from *Dasycladus vermicularis* suffers a shift when excreted to the seawater (Pérez-Rodríguez et al. 2001). Protection against UV radiation by phlorotannins in early developmental phases has been demonstrated in *Fucus* embryos and spores of *Laminaria* (Schoenwaelder et al. 2003; Henry and Van Alstyne 2004; Roleda et al. 2010). In the kelp *Lessonia spicata*, 24-h exposure to UV radiation increases the synthesis of phlorotannins, compared to control without UV (Fig. 18.3). In this species, the UV-mediated increase in phlorotannins can minimize photodamage of key physiological processes and cellular components, such as photosynthesis and DNA (Gómez and Huovinen 2010). Interestingly, differences between species, season, and morpho-functional processes have raised the question whether the photoprotective role of phlorotannins in brown algae can be regarded as a constitutive or inducible mechanism. For example, in *Lessonia*, the induction of phlorotannins by UV radiation has been shown to occur only during the period when sporophytes actively grow (Gómez and Huovinen 2010). However, in *Fucus vesiculosus*, no UV induction of soluble phlorotannins was found, which was related with a lack of upregulation of *pksIII* genes (Creis

et al. 2015). Overall, the few available studies point to a complex interplay between the induction of soluble phlorotannins enclosed in physodes and their subsequent deposition in the cell-wall matrix. Insoluble phlorotannins polymerized in the cell wall are regarded as primary UV-shielding substances (Gómez and Huovinen 2010), similar to cell-wall-bound phenolics reported in plants (Clarke and Robinson 2008). Moreover, photoprotection is conferred not only via intracellular accumulation of phlorotannins but also as a result of exudation to the surrounding water as has been suggested for adult thalli and propagules of the giant kelp *Macrocystis pyrifera* (Swanson and Druehl 2002).

18.4 Phlorotannins as Active Antioxidant Compounds

The formation of reactive oxygen species (ROS) is one of the primary expressions of stress in marine algae, but they can also act as signaling molecules in several cellular reactions (revised in Bischof and Rautenberger 2012). Phlorotannins are known to act, not only as photoprotective substances but also as highly efficient ROS scavengers (Nakai et al. 2006; Wang et al. 2009; Heffernan et al. 2014). Due to the presence of various interconnected rings (up to eight) in their chemical structure, phlorotannins are regarded as potent antioxidants scavenging different types of ROS, e.g., superoxide anions (O_2^-), peroxides, singlet oxygen (1O_2), and hydroxyl radicals ($^{\bullet}OH$) (Ahn et al. 2007; Koivikko et al. 2007). Thus, the hydroxyl groups present in phlorotannins act as reducing agents, hydrogen donors, and singlet oxygen (reviewed in Michalak 2006). It has been postulated that the relationship between increased levels of ROS and phlorotannin induction in brown algae can follow the methyl jasmonate signal transduction pathway, a plant defense-related pathway reported commonly in plants during high ROS production (Arnold et al. 2001; Küpper et al. 2009). The evidence gained during the last decades appears to indicate that operation of efficient and rapid ROS scavenging mechanisms based on phenols can be regarded as an important physiological adaptation in seaweeds when they are exposed to different environmental stressors, e.g., high solar irradiation, metal pollution, or high temperature (Aguilera et al. 2002; Contreras et al. 2009; Cruces et al. 2017).

18.4.1 Phlorotannins and UV-Induced Oxidative Stress

UV radiation is a primary factor inducing ROS in seaweeds, mostly by increasing the activity of peroxidases and NADPH oxidase (Mackerness et al. 2001). During exposure to high levels of UV radiation, the xanthophyll cycle is inhibited, which increases ROS production and impedes an effective dissipation of excess absorbed excitation energy of photosynthetically active radiation (PAR) (Dring 2005; Bischof et al. 2006b; Lesser 2012). Thus, the increased electron fluxes result in a direct

photoreduction of oxygen in the Mehler reaction in PSI and lower activity of Rubisco oxygenase, which exacerbate formation of ROS (e.g., superoxides) (Badger et al. 2000). In cold temperate/Arctic kelp gametophytes, formation of ROS could be demonstrated to occur predominantly in the peripheral cytoplasm or in plasmatic vesicles (Müller et al. 2012). Under UV stress, a relationship between the content of phlorotannins and antioxidant activity in various species of brown algae has been reported.

Temperature can modify the phlorotannin response to UV radiation. In the sub-Antarctic *Lessonia spicata*, *Durvillaea antarctica*, and *Macrocystis pyrifera* from the coast of Chile, a rapid induction of soluble phlorotannins triggered by UV radiation ameliorated the effects of oxidative stress on photochemical processes after short-term thermal stress (Cruces et al. 2012). Interestingly, under elevated temperatures >20 °C, the UV induction of phlorotannins ceases, and lipid peroxidation increases in *D. antarctica* and *L. spicata*, suggesting that the ROS scavenging potential of these sub-Antarctic species has a geographic component associated with prevalent UV levels and temperature (Cruces et al. 2013). This can have important consequences for stress tolerance in species living at the limits of their geographic distribution or those exposed to changing conditions of temperature and solar radiation (e.g., polar, intertidal species). In fact, at a molecular level, oxidative stress caused by UV radiation in the Arctic kelp *Saccharina latissima* is higher at 2 °C, which is reflected in upregulation of genes encoding for different ROS defense mechanisms, especially antioxidant enzymes, but not phlorotannins (Heinrich et al. 2015). On the other hand, UV radiation does not directly regulate the expression of genes involved in phlorotannin metabolism in the intertidal *Fucus vesiculosus*, suggesting that UV induction of these substances relies on other processes or their accumulation represents a constitutive metabolic strategy (Creis et al. 2015). Because phlorotannins act also as primary, structural cell components, their accumulation depends on cellular cycles and biomass formation. Hence, the constitutive nature of phlorotannins confers side-by-side advantages to brown algae under multiples stress factors, including UV radiation (Arnold and Targett 2003; Gómez and Huovinen 2010).

18.4.2 Phlorotannins and Their Interaction with Metals

Although the relationship between phlorotannins of brown algae and metals is not fully understood, increasing evidence indicates that metal tolerance of seaweeds can be associated with both internal and external metal-complexing ligands (Andrade et al. 2010; Connan and Stengel 2011). Decreased levels of soluble phenolic compounds in seaweeds (e.g., *Scytosiphon lomentaria* and *Ulva compressa*) have been reported in copper-impacted sites (Ratkevicius et al. 2003; Contreras et al. 2005). Metals are redox active and also participate in many reactions generating ROS. The importance of phenolic compounds as key antioxidant agents during metal exposure has been recognized in plants (reviewed by Sakihama et al. 2002). UV radiation is

known to induce or enhance the toxicity of certain organic contaminants (phototoxicity) (Huovinen et al. 2001), and the presence of various metals has been reported to have antagonistic effects on seaweeds (Andrade et al. 2006). In copper-impacted areas, seaweeds (e.g. *Ulva compressa*) have been shown to develop oxidative stress, and decreased levels of soluble phenolic compounds have been reported (Ratkevicius et al. 2003; Contreras et al. 2005). Adverse effects of copper in the brown alga *Laminaria digitata* were buffered by protective mechanisms regulated by lipid peroxide derivatives (Ritter et al. 2008). Proteins potentially involved in the control of copper-mediated oxidative stress in the brown alga *Scytosiphon gracilis* were identified recently (Contreras et al. 2010). Species-specific antioxidant activity of the soluble phlorotannins and its response to environmental stress (UV radiation, metals) has been shown in three Pacific kelps (Huovinen et al. 2010). Here, inorganic nitrogen was shown to mitigate the adverse effects of copper: the impact of the interaction of copper, nitrate, and UV radiation was species-specific, *Lessonia spicata* showing the strongest responses in photosynthetic activity and *Durvillaea antarctica* the strongest response in phlorotannins and their antioxidant activity. *Macrocystis pyrifera* accumulated threefold more copper in its tissues than the other kelps, but its photosynthetic activity was twofold less inhibited by copper than in *D. antarctica*, suggesting higher metal tolerance of *M. pyrifera*, which was partly explained by the decreased accumulation of copper in the algal tissues in the presence of nitrate (Huovinen et al. 2010).

Whether phlorotannins react increasing their ROS scavenging activity after exposure to metals is not well known. When algae are exposed to metal stress, increased exudation of organic compounds, including probably phlorotannins, may retain free metals in form of extracellular complexing ligands. On the other hand, detoxification of intracellular metals via algal exudates may also increase (Andrade et al. 2010). *Lessonia spicata* from uncontaminated sites has been shown to have capacity to rapidly respond to copper exposure by producing organic ligands that, due to their complexing capacity in the water, can rapidly attenuate the level of labile copper (Andrade et al. 2010), thus affecting its bioavailability.

18.5 Phlorotannins in Antarctic Seaweeds

An important feature of various endemic Antarctic brown algae is their high content of phlorotannins. Different studies have reported total phlorotannin contents in Antarctic brown algae ranging between 1% and 12% DW. In the case of insoluble phlorotannins, values are between 1% and 5% DW (Table 18.1). Although some cold-temperate genera (e.g., *Fucus*, *Ascophyllum*) can contain high concentrations of phlorotannins (>10% DW), normally the maximal values detected in Antarctic brown algae are higher than most of reported values from temperate, cold-temperate, and Arctic species (Connan et al. 2004; Dubois and Iken 2012; Cruces et al. 2013; Generalić-Mekinić et al. 2019). The high concentrations of phlorotannins in some endemic Antarctic brown algae can be also evidenced by the abundance of physodes

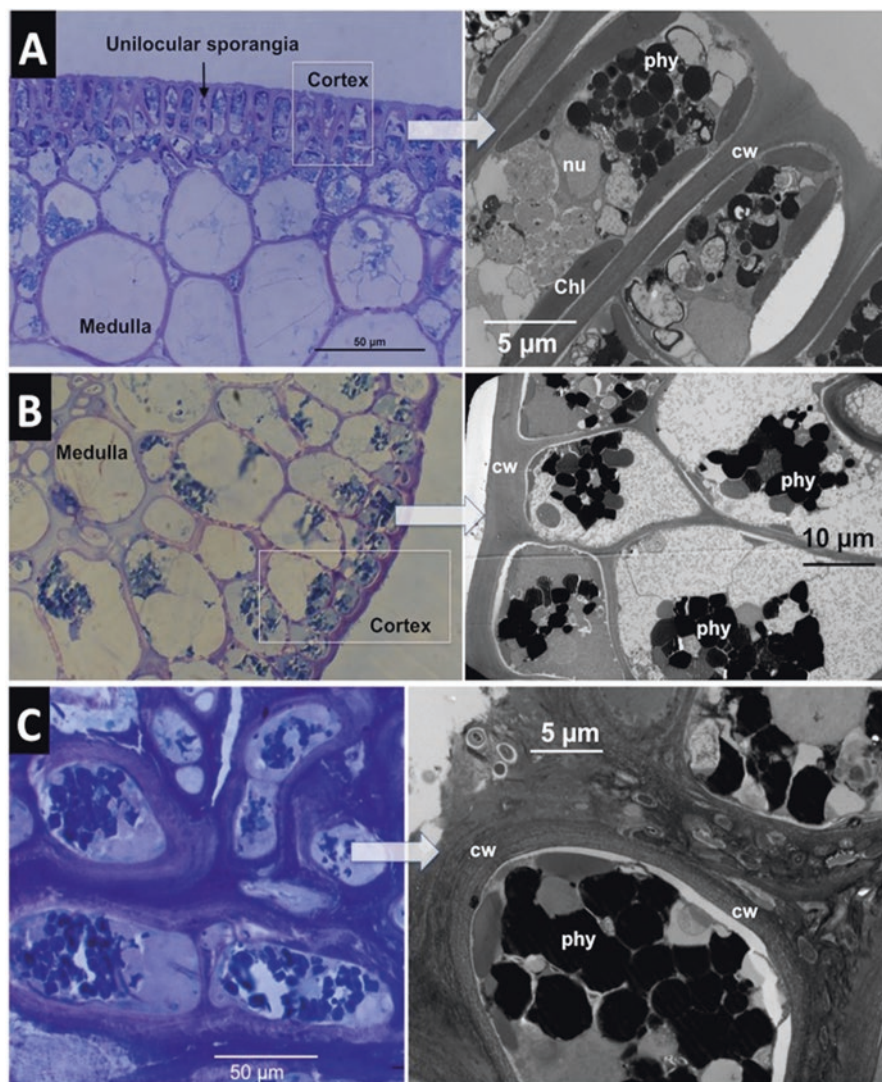


Fig. 18.4 Ultrastructure and localization of phlorotannin-containing physodes in Antarctic brown algae. (a) *Desmarestia anceps*; (b) *Phaeurus antarcticus*; (c) *Halopteris obovata*. Left: cross sections stained with toluidine blue; right: transmission electron microscopy of cortical cells indicating the presence of physodes (*phy*). Cell wall (*cw*) and chloroplasts (*chl*) are indicated

in the outer cell layers (Fig. 18.4). Herbivory is among the factors that may contribute to these high levels of phlorotannins (see Chap. 17 in this volume); however, other intrinsic factors related to biomass formation can also play an important role. Whether these high phlorotannin levels found in endemic brown algae living between 5 and 30 m depth can also be associated with photoprotection against high

solar radiation has also been evaluated (Gómez and Huovinen 2015). In the following sections of the present chapter, the variability in the contents of phlorotannins is revised in the context of abiotic stress.

18.5.1 Depth Patterns in Phlorotannin Contents

The vertical distribution of endemic Antarctic brown algae can range from 1 to 2 m down to 40 m or greater depths. This broad distribution is related with a suite of photobiological adaptations operating in a range of different light fields (see Chap. 11 in this volume). Although Antarctic seaweeds are normally not exposed to high UV levels, seasonal and oceanographic conditions can increase the eventual incidence of harmful irradiances (the biological impact of this factor on different processes related with algal distribution is revised in Chap. 11 by Gómez and Huovinen). The hypothesis that phlorotannins of Antarctic seaweeds can also be related with the light acclimation strategies has been tested in algae collected at different depths (Fairhead et al. 2005a; Huovinen and Gómez 2013; Gómez and Huovinen 2015). In eight brown algae collected along a depth gradient in King George Island, species such as *Desmarestia anceps*, *Cystosphaera jacquinotii*, and *Himantothallus grandifolius* collected at depths >20 m showed the highest phlorotannin concentrations, in contrast to shallow water or intertidal species such as *Adenocystis utricularis* or *Ascoseira mirabilis*, which in general had the lowest values (Huovinen and Gómez 2013). However, when intraspecific variability of phlorotannins is examined, this pattern can be different. In fact, Gómez and Huovinen (2015) analyzed the contents of phlorotannins in conspecifics of *Ascoseira mirabilis*, *Desmarestia anceps*, *D. menziesii* and *Himantothallus grandifolius* collected from 5/10, 20 to 30 m at King George Island, and found that variation with depth was species-specific. For example, in *A. mirabilis*, no changes with depth were detected, while in *D. anceps* and *D. menziesii*, values increased in algae collected at 10 m depth compared to 20 or 30 m. Similar results have been reported in *D. anceps* from Anvers Island, West Antarctic Peninsula, where higher phlorotannin contents were measured in shallower locations (3–12 m) compared to samples collected between 18 and 30 m depth (Fairhead et al. 2005a). Although many factors can preclude a conclusive comparison between different studies (e.g., time of collection, study site, and the characteristics of the depth gradient or differences in depths between samples), the results appear to indicate that (a) endemic Antarctic brown algae from depth >20 m in general show constitutively high levels of phlorotannins, (b) phlorotannin contents and their vertical variability mirror differences in life adaptations developed to cope with multiple abiotic or biotic variables, and (c) phlorotannins form part of a trade-off between shade adaptation marked by high photosynthetic efficiencies at low light and tolerance to high solar stress. Thus, phlorotannins act as multifunctional substances that can be “mobilized” in any situation that poses a threat to the algae (Gómez and Huovinen 2015) (see Sect. 18.5.3).

18.5.2 *Phlorotannin Allocation in Antarctic Seaweeds*

Although the Antarctic is devoid of kelps, which resemble plants in being structurally complex, many Antarctic Desmarestiales and *Ascoseira* and *Cystosphaera* show a complex thallus anatomy with morpho-functional processes analogous to those described in Laminariales and Fucales (see Chap. 11 in this volume). In this context, it has been commonly observed that concentrations of phlorotannins vary strongly among different thallus parts (Van Alstyne et al. 1999; Connan et al. 2004; Iken et al. 2007), stimulating researchers to propose diverse hypothesis explaining whether this observed variability is related with functional processes at an organismal level. Whether the unequal allocation of phlorotannins to different thallus regions is related with putative benefits for the alga, e.g., protection of metabolic performance, reproductive output, or, in general, to guarantee the algal fitness during environmental stress, is a relevant question. The optimal defense theory (ODM; Rhoades 1979) is one of the ecological models used to explain the differential distribution of phlorotannins in the brown algal thalli, suggesting that chemical defenses are produced in direct proportion to the risk, i.e., the phenolic compounds would be produced at a direct expense of other functions (Pavia et al. 2002). High concentrations of phlorotannins may be expected when an environmental pressure, e.g., grazing, is high (inducible response) on thallus parts that make an important contribution to the whole fitness (e.g., meristematic or reproductive regions) or during seasonal periods when algae are especially vulnerable. Results in *Fucus* and *Ecklonia* (Steinberg 1985; Yates and Peckol 1993) and the sub-Antarctic kelps *Lessonia spicata* and *Macrocystis pyrifera* (Pansch et al. 2008) indicate that phlorotannins could vary as predicted by the ODT. As has been reported for *Ascophyllum nodosum*, production of phlorotannins can be highly costly at the expense of growth (Pavia et al. 1999). Thereby, it has been proposed that due to these costs, synthesis and accumulation of phenolics could indicate inducible rather than constitutive defenses (Rhoades 1979), which has been confirmed in some studies of simulated herbivory (Lüder and Clayton 2004). However, studies carried out in some Antarctic species indicate that phlorotannin allocation not necessarily confers chemical defense consistent with the ODM assumptions. For example, regarding the “value” of different thallus parts in relation with perennial and annual growth strategies, *D. anceps* did not show differences in phlorotannin contents between thallus parts (Fairhead et al. 2005a; Iken et al. 2007); however, there were marked differences in the toughness, and the chemical defenses in primary stems/stipes were much higher than the laterals supporting the ODT model (Fairhead et al. 2005b). In contrast, *D. menziesii* and *Ascoseira mirabilis* had higher phlorotannin concentrations in the holdfasts compared to the branch or lamina regions. Due to that holdfasts were regarded here as the most valuable thallus part conferring attachment, the patterns in these species appear to meet well the ODT (Iken et al. 2007). It must be emphasized that the deterrent role of Antarctic phlorotannins against grazers and microbia or as antifouling agents is species specific and probably depends on the type of predominant phlorotannins and other not well-known qualitative properties of this

compounds (reviewed in Iken et al. 2009). The results agree with longitudinal profiles determined in the cold-temperate kelps *Laminaria hyperborea* and *Laminaria digitata* (Connan et al. 2006) and *Lessonia spicata* (Gómez et al. 2016), where valuable regions and basal parts such as haptera or holdfasts and meristematic tissues allocated the highest phlorotannins compared to the fronds, which can be regarded as transient structures. These patterns can be associated with various longitudinal profiles of physiological performance normally described for various kelps (Van Alstyne et al. 1999; Gómez et al. 2005; Gruber et al. 2011).

In the case of photoprotective responses, it could be reasonable to argue that valuable thallus regions, e.g., reproductive tissues, should be protected when they are exposed to UV radiation (Holzinger et al. 2011). This hypothesis has also been tested in two Antarctic brown algae by exposing reproductive and vegetative thallus pieces to UV radiation during a short-term period (Huovinen and Gómez 2015). In the brown alga *Cystosphaera jacquinotii*, the reproductive structures (receptacles containing conceptacles) showed higher UV tolerance than its vegetative blades, whereas in *Ascoseira mirabilis*, high UV tolerance was demonstrated in both vegetative and reproductive tissues. Interestingly, the reproductive structures of both species of brown algae had higher levels of soluble phlorotannins than the vegetative tissues, and thus, allocation and proportions of soluble and insoluble, cell-wall-bound phlorotannins could be related with the observed patterns of UV tolerance of the different tissues. Observations of tissue cross sections under violet-blue light excitation using epifluorescence microscopy confirmed a high allocation of phenolic compounds (as blue autofluorescence) in *C. jacquinotii*, especially in its reproductive structures (Fig. 18.5a). The study is among the first approaches to address the defense strategies that Antarctic macroalgae exploit to protect their reproductive structures. It is likely that the allocation of chemical defenses and UV-absorbing compounds in reproductive tissues is a widespread strategy to ensure the viability of spores and gametes during their maturation. For example, blue autofluorescence, indicating the presence of phenolics compounds in reproductive tissues (carporangia) of the Antarctic red algae *Trematocarpus antarcticus* (Fig. 18.5b), suggests that not only phlorotannins but also other phenolics can be allocated providing protection to reproductive tissues.

It has been suggested that phlorotannins can be remobilized between tissues with different metabolic demand and age (Arnold and Targett 2000). In Laminariales, compounds as mannitol, amino acids, and other low-molecular-weight compounds are transported through specialized cells to power meristematic growth through translocation processes (Küppers and Kremer 1978; Gómez and Huovinen 2012). Since phlorotannins are structural components in cells, it may be intuitively suggested that these compounds or some key precursors may be mobilized along the thallus. The abundance of low-molecular-weight phlorotannins (<1200 Da) in various species of brown algae (Steevensz et al. 2012) supports also the idea that these compounds might be rapidly remobilized. For example, accumulation of phlorotannins in response to artificial wounding in the kelp *Ecklonia radiata*, including the presence of physodes in medullary sieve elements (Lüder and Clayton 2004), suggests that these compounds can be “transported” along the thallus. In fact, some

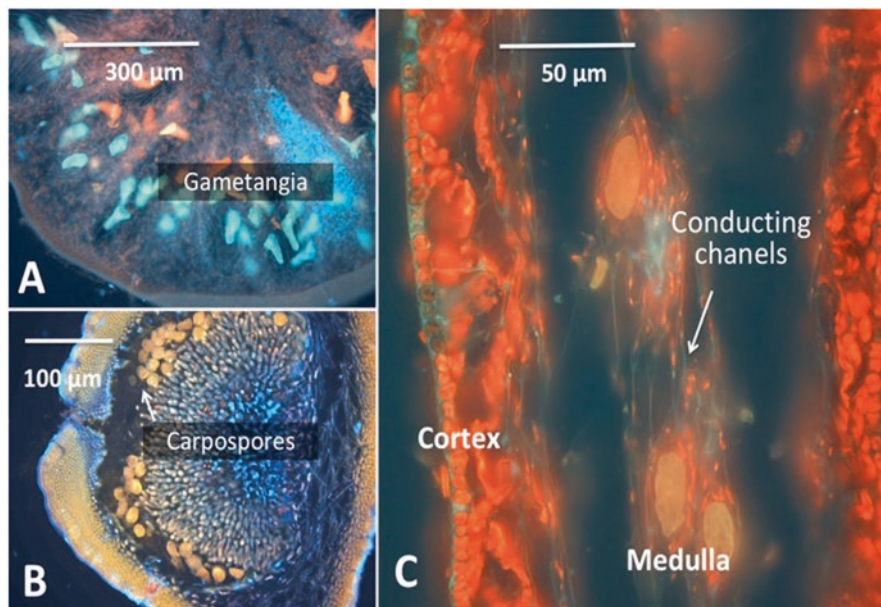


Fig. 18.5 Blue autofluorescence of phenolic compounds under violet-blue light excitation in (a) reproductive receptacles of the brown alga *Cystosphaera jacquintii* and (b) carposporangium in the Antarctic red alga *Trematocarpus antarcticus*; (c) cross section of non-reproductive lamina of brown alga *Ascoseira mirabilis* indicating presence of phenolic compounds in medullar “conducting channels”

Antarctic species such as *Ascoseira mirabilis* appear to have the anatomical prerequisites as this alga shows “conducting channels” in its medulla (Fig. 18.5c), which are suggested to have putative functions in remobilization of substances (Clayton and Ashburner 1990). Overall, phlorotannins are important structural elements in the algal thallus, and since growth is localized in specific regions, a trade-off between phlorotannin synthesis, mobilization, and growth might be defined in these species in a similar way as in terrestrial vascular plants.

18.5.3 Phlorotannins in Response to UV Radiation

Experimental evidence has pointed to a relatively high tolerance of Antarctic seaweeds to UV radiation in the short-term, which at least partly can be related to their chemical defense mechanism based on phenolic substances. Indeed, Antarctic algae exposed for 2 h to UV radiation at 2 °C showed very low inhibition of photosynthesis measured as maximal quantum yield of fluorescence (F_v/F_m), which can reach up to 35% in algae collected from depth >20 m. Even in algae growing at 30 m, inhibition of chlorophyll fluorescence did not exceed 10–15% (Huovinen and Gómez

2013). In the case of the brown algae, almost all have high levels of phlorotannins, which due to their UV-absorbing characteristics are the main candidates conferring photoprotection in these species (Huovinen and Gómez 2013; Gómez and Huovinen 2015; Núñez-Pons et al. 2018). However, testing these properties experimentally is not an easy task. In fact, manipulative studies conducted in algae attaining high concentrations of phenols, e.g., *Desmarestia anceps*, have not demonstrated induction in phlorotannins in response to UV (Fairhead et al. 2006; Gómez and Huovinen 2015; Flores-Molina et al. 2016). In contrast, some species with relatively low concentrations, such as *Ascoseira mirabilis*, show a slightly UV-mediated induction of soluble phlorotannins (Rautenberger et al. 2015). As this species is normally found at shallower depths (1–10 m), the results suggest that it can become exposed to harmful solar radiation in summer, thus activating the synthesis of phlorotannins. In all, photoprotection against excess solar radiation, e.g., via UV shielding, is a collateral function in Antarctic seaweeds as these molecules form part of an integral defense machinery operating in response to multiple stressors in the polar environment such as herbivores, antifouling, and changes in temperature or simply they are synthesized to supply of structural elements during cell growth (as insoluble phlorotannins). These multiple functional roles are explained by their high antioxidant capacity, the most important chemical property of phlorotannins. In fact, soluble phlorotannins have been positively correlated with the high antioxidant potential determined in extracts of various species of Antarctic brown algae. This positive correlation is observed in algae exposed to different conditions of UV radiation and temperatures (Gómez and Huovinen 2015; Flores-Molina et al. 2016). Interestingly, it has been demonstrated that UV effects on photosynthesis in Antarctic macroalgae are modified by temperature: when algae are incubated at 7 °C, i.e., 5 °C above the field temperature, inhibition of photosynthesis decreases, and recovery increases, suggesting that, e.g., the PSII repair cycle is more effective at elevated temperature resulting in a higher UV tolerance, at least in the short-term (Rautenberger et al. 2015). However, the relationship between the phlorotannin contents and the antioxidant potential of extracts does not change with temperature (Fig. 18.6), reinforcing the idea that in these species, phlorotannins are not UV-inducible compounds. This raises questions related to the role of these compounds and their physiological consequences under changing environmental conditions. For example, it is known that during oxidative stress, Antarctic brown algae can activate their enzymatic machinery (e.g., superoxide dismutase, SOD), whose efficiency varies in response to environmental gradients (Bischof and Rautenberger 2012). Thus, the operation of complementary mechanisms of ROS detoxifying less affected by, e.g., temperature or UV radiation could be favored. In this context, it has been reported recently that under high solar stress conditions, algae display a suite of complementary and consecutive protective mechanisms based on energy dissipative downregulation of photosynthesis, rapid pigment acclimation and PSII repair mechanisms, synthesis of phenolics with specific UV absorption characteristics, and complementary ROS scavenging mediated by antioxidant enzymes and phenols (Cruces et al. 2017).

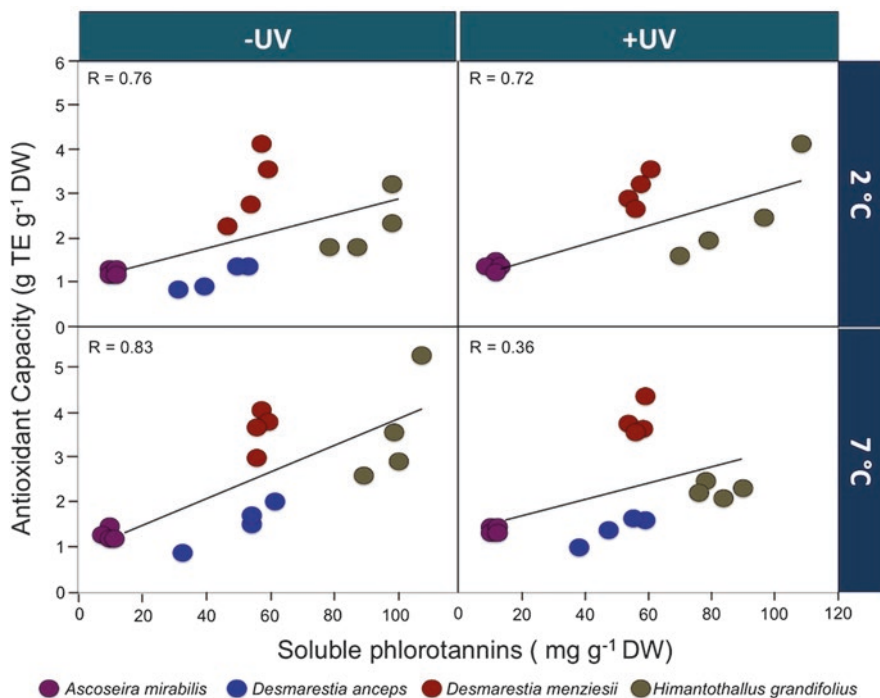


Fig. 18.6 Relationship between phlorotannin content and antioxidant activity of extracts from four Antarctic brown algae after exposure to UV radiation under different temperatures (Adapted from Rautenberger et al. 2015)

18.6 Concluding Remarks

Antarctic macroalgae are equipped with a suite of anti-stress mechanisms to cope with the harsh polar environment, probably much more sophisticated and efficient than previously thought. However, in the case of the putative roles of phlorotannins in stress tolerance, many gaps still persist, especially those related with their synthesis, the action of different forms of phlorotannins, and their turnover and regulation. In the case of Antarctic algae, no data on gene expression exist, which precludes an understanding of crucial aspects related with their multifunctional roles.

In Antarctic brown algae, processes related with synthesis and functional dynamics of phlorotannins take place under constantly low temperatures of 0–2 °C, and phlorotannin induction can be sensitive to changes in temperate and cold-temperate species. This raises questions related with the effects of shifts in temperature on the anti-stress properties of these substances in endemic Antarctic algae, where induction has not been reported.

Finally, the role of phlorotannins in response to new and emergent stressors, e.g., pollutants and acidification (OA), has been very little studied in Antarctic algae. Although recent experimental essays carried out in *Desmarestia anceps* and *D.*

menziesii indicated that a combination of low pH and elevated temperature does not result in marked effects in phlorotannin content, the shifts can have important and not well-understood ecophysiological consequences (Schoenrock et al. 2015).

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