Antarctic Science 12 (4): 418–424 (2000) © British Antarctic Survey Printed in the United Kingdom

Ice-active substances associated with Antarctic freshwater and terrestrial photosynthetic organisms

JAMES A. RAYMOND[1]* and CHRISTIAN H. FRITSEN[2]

[1]*Department of Biological Sciences, University of Nevada, Las Vegas, NV 89154, USA*
[2]*Division of Earth and Ecosystem Sciences, Desert Research Institute, Reno, NV 89512, USA*
**raymond@nevada.edu*

Abstract: Macromolecular substances that cause pitting and other modifications of growing ice crystals were found to be associated with cyanobacterial mats, eukaryotic algae and mosses from Ross Island and the McMurdo Dry Valleys, Antarctica. Ice-pitting activities were largely retained by dialysis membranes with molecular weight cut-offs of up to 300 kDa. Unlike most aqueous solutes, the ice-active molecules were not excluded from the ice phase during freezing. The ice-pitting activities of each of the samples tested was destroyed by exposure to temperatures between 45 and 65°C, suggesting that they have a protein component. Ice-active substances were not found in cyanobacteria or mosses from temperate climates, but ice-activity was found to be associated with mosses from cold habitats in North America. Although the function of the ice-active substances is not known, their apparent confinement to cold environments suggests that they have a cryoprotective role.

Received 25 April 2000, accepted 27 July 2000

Key words: cyanobacteria, ice-active substances, mosses, *Prasiola*

Introduction

Terrestrial and freshwater photosynthetic organisms – cyanobacteria, eukaryotic algae, lichens and mosses – grow well in many parts of Antarctica (e.g. Lamb 1970, Broady 1996, McKnight *et al.* 1998, Hawes *et al.* 1999) despite their exposure to extreme low temperatures for a large part of the year and frequent freeze-thaw cycles in the short growing season. Various studies have addressed freezing tolerance and freezing tolerance mechanisms in Antarctic cyanobacteria (Holm-Hansen 1963, Becker 1982, Davey 1989), the Antarctic alga *Prasiola* (Becker 1982, Davey 1989, Jacob *et al.* 1992, Jackson & Seppelt 1995, Bock *et al.* 1996), and polar mosses (Sakai & Larcher 1987, Tearle 1987, Lovelock *et al.* 1995). Although a thermal hysteresis protein has been reported in one species of moss from North America (Duman & Olsen 1993), there have been no reports of molecules that specifically interact with ice in polar plants or freshwater algae.

In the Antarctic marine environment, two major types of ice-active molecules are known. These include protein and glycoprotein fish antifreezes (Cheng & DeVries 1991) and protein-containing macromolecules that are associated with sea ice diatoms (Raymond *et al.* 1994, Raymond 2000). Both types of molecules retard the growth of specific faces of ice crystals, which is an indication that they bind to these faces (Buckley 1952). While the fish antifreezes lower the freezing point and thus protect the fish from freezing, the diatom-associated molecules have little effect on the freezing point. However, it seems likely that these molecules have some role in polar habitats, e.g. as an adhesive for diatoms that attach to ice or as a cryoprotectant, since such molecules have not been found in non-polar diatoms.

The present study was undertaken to determine whether various Antarctic freshwater and terrestrial photosynthetic organisms – cyanobacteria, eukaryotic algae and mosses – might also produce such molecules. Here we demonstrate that ice-active substances (IASs) are associated with a variety of such organisms and describe some of their properties.

Materials and methods

Materials

Samples were collected within a 100 km radius of McMurdo station, Antarctica, including areas in the Miers, Trough, Taylor and Wright valleys, Granite Harbour, and several points on Ross Island (Hut Point, Cape Evans, Cape Crozier) during the months October–December 1998 and January–February 2000.

Light-coloured cyanobacterial mat samples were collected at the edges of two small melt ponds at Cape Evans, Ross Island (*c.* 200 m south of Scott's hut). These mats had grown in shallow water, but at the time of their collection, they were partially dried. Similar mats at a nearby pond (Algal Pond) at Cape Evans were largely composed of *Oscillatoria*, *Phormidium* and *Nostoc* (Goldman *et al.* 1972) and resembled the *Phormidium/Oscillatoria* mats in the Taylor Valley described by McKnight *et al.* (1998). Dark mat samples, largely *Nostoc* sp., were collected from the edges of glacier melt streams at lakes Bonney and Fryxell in the Taylor Valley, and were mostly in a semi-dried state. The eukaryotic alga

Prasiola sp. was collected from depressions in rocks, where melt water had collected, along the shore at Botany Bay (Granite Harbour). Both damp and dry samples were collected. Moss samples included *Bryum* spp. collected from glacial melt streams at lakes Fryxell and Miers, and *Bryum argenteum* Hedw. collected from Botany Bay (moss identifications by Dr L. Stark).

Additional cyanobacteria samples (*Nostoc* spp., *Anabaena* sp. and mixed soil cyanobacteria) and moss samples (*Syntrichia caninervis* Mitt., *Syntrichia norvegica* Web., *Syntrichia* sp., *Bryum* sp., *Grimmia anodon* Bruch & Schimp., *Grimmia* sp., and *Didymodon tophaceus* (Brid.) Lisa) were collected from natural habitats in the south-western United States. Some of these samples were stored at room temperature in a dried state, or at 4°C in a semihydrated state, for 6–9 months. One of the dried moss samples (the only one of the dried samples that was collected in a cold environment) had good ice-pitting activity, so this treatment was not thought to cause substantial degradation of activity.

All samples were ground with a mortar and pestle, with *c.* 2 ml buffer (0.05 M NaCl, 15 mM Tris, pH 8.0; Tris-NaCl buffer) per gram of wet material to make a thick slurry. For the moss samples, approximately equal amounts of leaves and roots were used for the homogenate. The homogenates were centrifuged at 14 000 rpm for 5 min. The supernatants were used for subsequent analyses.

Measurement of ice activity

Ice activity was measured by immersing a single crystal of ice in the solution to be assayed at a temperature *c.* 0.1C° below its freezing point. The degree of pitting that appeared on the ice basal surface was viewed with a dissecting microscope at magnifications between 10x and 40x. The temperature bath and the method for preparing ice single crystals are described in Raymond *et al.* (1989). For quantitative purposes, activities were visually rated on a scale of 0 to 10, after a period of *c.* 30 min (shorter times were used for active samples). Activities at various points on this scale were as follows: 0 = no activity, 2 = weak activity with pitting of 10% or less of the basal plane of an ice crystal, 4 = moderate activity with 60% pitting of the basal plane, 6 = strong activity with 90% pitting, 8 = very strong activity with 100% pitting with small pits (< 75 mm in diameter), 10 = intense activity, as shown by complete coverage of the basal plane with small pits within a few minutes.

Molecular weight

Initial attempts to purify the IASs from cyanobacterial homogenates with ion exchange (Whatman CE-52) and gel permeation (Sephadex G-75) chromotographies were not successful as they resulted in broad bands of activity. As an alternate means of characterizing the IASs, their approximate sizes were determined with dialysis experiments. Approximately 1 ml samples were dialysed in 25, 50, 100, and 300 kDa MWCO DispoDialyser (cellulose ester) dialysis tubes (Spectrum) against *c.* 600 ml 0.1 M NaCl, 10 mM Tris, pH 8.0 overnight (*c.* 20 h), with stirring, in a cold room, and then their activities were assayed. According to the manufacturer, the approximate pore diameters of these dialysis membranes are 0.002 mm, 0.005 mm, 0.01 mm, and 0.02 mm, respectively.

Thermal stability

To determine the thermal stability of the IASs, active supernatants of homogenates of each of the samples were immersed in water at temperatures ranging from 35°C to 100°C for 5 min, cooled and then assayed for ice-pitting activity.

Ice–liquid partition

Approximately 15 ml of ice-active supernatant was prepared from homogenates of each of the samples. The osmolalities of the supernatants were adjusted to *c.* 200 mOsmol kg^{-1} with NaCl crystals. The samples were divided into three equal parts in 10 ml centrifuge tubes with screw caps, cooled in a freezer until ice began to form, transferred to an ethylene glycol bath at -2.0°C and left overnight to freeze. These conditions led to a freezing of *c.* 80% of the water, at which point the ice and liquid phases would be in equilibrium. The outside of the tubes were rinsed in cold brine to remove the glycol, and then centrifuged upside down in a refrigerated centrifuge (rotor temperature about -3°C) at *c.* 2000 rpm for 2 min. The centrifugation forced the liquid part of the sample into the cap. The tube was removed from the centrifuge, and while holding it in an upside down position, the cap was removed. The liquid portion, containing impurity molecules and most of the salt, was removed from the cap. The centrifugation process was repeated (about three more times with slight increases in speed and time) until no further liquid was expelled from the remaining ice in the tube. The ice fraction was melted, and then both the ice and liquid fractions were brought to an osmolality of about 200 mOsmol kg^{-1} (by adding NaCl and dialysis, respectively) and then assayed for ice activity as described above. The liquid fractions were dialysed for about 30 min in 12–14 kDa MWCO dialysis tubing in Tris-NaCl buffer. The UV absorption of the solutions at 260 nm was used as an index of the amount of impurities (e.g., proteins, nucleic acids, pigments) that were present. The ice and liquid fractions were diluted by factors of about 10 and 150, respectively, to obtain measurable readings. Osmolalities were measured with a Wescor model 5500 vapour pressure osmometer.

Results

Ice activity

Ice activities were determined for light-coloured cyanobacterial mats (mostly *Phormidium*), dark-coloured cyanobacterial mats (mostly *Nostoc*), *Prasiola*, and moss samples from several locations around McMurdo Sound. The light-coloured cyanobacterial samples, which included both wet and dry samples, showed a wide range of activities, ranging from none (ice activity score of 0) to strong activity (ice activity score of 10). The mean ice activity (± s d) of 75 samples tested from various locations and habitats was 4.1 ± 3.3. The dark mat samples tended to be more active (5.8 ± 2.4, $n = 21$) (*t*-Test, $P < 0.02$). There was no clear relation between the ice activity of the mat samples and their state of hydration. On the other hand, all of the *Prasiola* samples examined, both wet and dry, showed good activity (7.5 ± 1.1, $n = 6$), and all of the moss samples examined showed good activity (7.2 ± 1.7, $n = 12$).

Examples of ice pitting activities of ice-active supernatants from homogenates of cyanobacterial mats, *Prasiola* sp. and a moss, *Bryum* sp. are shown in Fig. 1. In each of the samples, ice activity typically began in the form of small pits on the ice surface, and these were eventually transformed into more complex patterns, such as shown in Fig. 1c. The pitting was the result of growth of the basal plane of ice around the nascent pits, rather than through excavation of the pits.

Molecular weight

Supernatants of homogenized samples having an initial ice activity of 10 retained their activity (final activity values *c.* 6–9) following dialysis in tubing with molecular weight cut-offs (MWCOs) ranging from 25 kDa to 300 kDa (Fig. 2). The activities of the samples decreased only slightly with increasing MWCO, suggesting that the IASs are mostly larger than 300 kDa.

Ice-binding properties

Solutes in aqueous solutions are normally excluded from the ice phase during freezing. To determine the extent to which the IASs are excluded from, or retained in, growing ice, supernatants with high ice activities were prepared from homogenates and partially frozen, and then the liquid and ice phases were separated by centrifugation. As shown in Fig. 3a, the concentration of solute impurities, expressed by the UV absorbance at 260 nm, was about one order of magnitude greater in the liquid phases of the four samples than in the ice phases, which was essentially as expected. Similar differences were observed in the osmolalities of the two phases, which are mainly reflections of the concentrations of small molecular weight substances such as NaCl. In contrast to the strong exclusion of UV-absorbing materials and salts from the ice phase, the ice pitting activities of the ice and liquid fractions of the four samples were essentially the same (Fig. 3b),

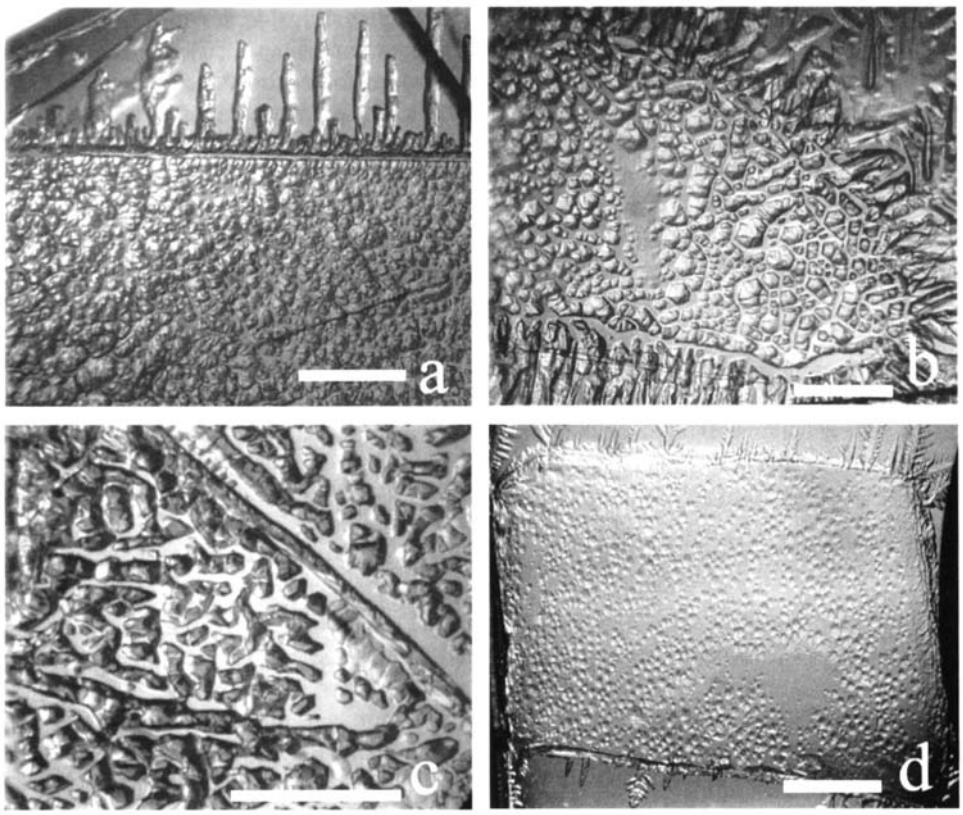

Fig. 1. Growth of ice single crystals placed in solutions of algal and moss extracts containing ice-active substances. **a.** Cyanobacterial mat (mostly *Phormidium*), **b.** cyanobacterial mat (mostly *Nostoc*), **c.** *Prasiola* sp., **d.** *Bryum* sp. (moss). Ice basal plane is parallel to plane of figure. Roughness is due to pitting and other irregular depressions in the basal plane. Pitting does not occur in the absence of ice-active substances. Scale bars = 1 mm.

https://doi.org/10.1017/S0954102000000493 Published online by Cambridge University Press

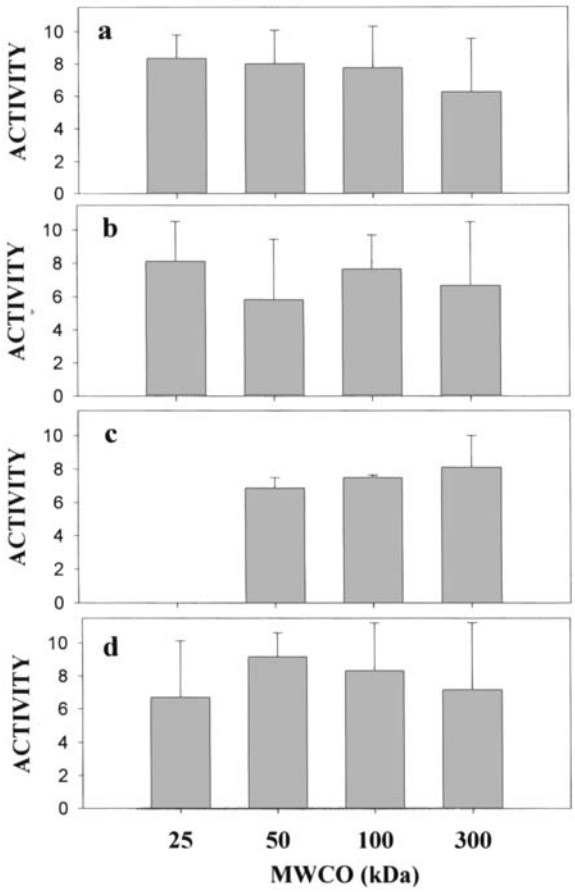

Fig. 2. Ice-pitting activity of extracts of algal and moss tissues after dialysis in tubing with various nominal molecular weight cut-offs (MWCO). **a.** Cyanobacterial mat containing mostly *Phormidium* (n = 3–5 for each assay), **b.** cyanobacterial mat containing mostly *Nostoc* (n = 3–6), **c.** *Prasiola* sp. (n = 2), **d.** *Bryum* sp. (moss) (n = 3–5). Error bars show 1 s d, except for c, in which bars show one-half of range.

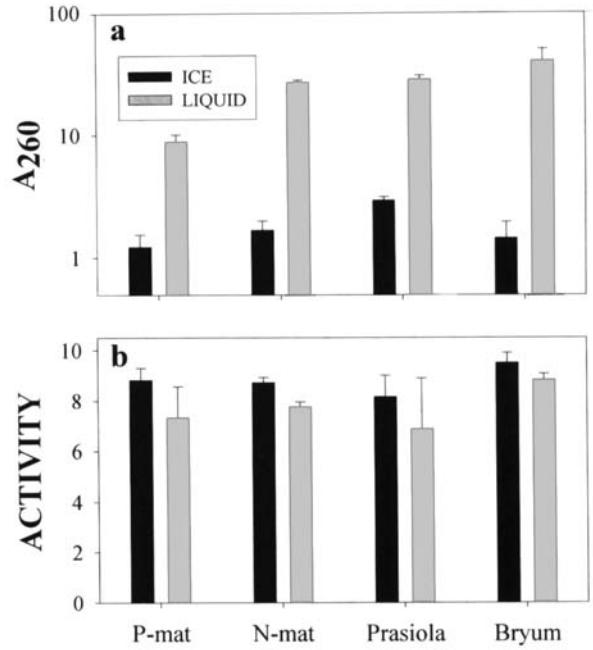

Fig. 3. Distributions of impurities and ice-active substances between ice fractions (black bars) and liquid fractions (grey bars) of *c.* 80% frozen solutions containing ice-active substances. **a.** Amounts of impurities, expressed as UV absorption at 260 nm (logarithmic scale). **b.** Ice-pitting activities. P-mat = cyanobacterial mat containing mostly *Phormidium*; N-mat = cyanobacterial mat containing mostly *Nostoc*, Prasiola = *Prasiola* sp.; Bryum = *Bryum* sp. Bars show mean values of 3 samples all derived from the same homogenate; error bars show one s d.

indicating that the IASs were not being excluded from the growing ice. Because the volumes of the ice fractions were 4 to 5 times larger than the liquid fractions, most of the total activity remained in the ice phases.

Thermal stability

The ice pitting activities of all four of the samples were destroyed by short (5 min) exposures to elevated temperatures (Fig. 4). The temperatures at which 50% of the activity was lost were about 45°C and 65°C for the light and dark cyanobacterial samples, *c.* 45°C for *Prasiola* samples and about 53°C for the *Bryum* samples.

Other organisms

Four unidentified lichens were collected from the rocky shore at Botany Bay in January. Orange, grey and black lichens showed very weak to moderate activity and one yellow lichen showed good activity. A *Chlorella*-like unicellular alga (identified by Dr L. Goff) obtained from a puddle at Cape Crozier in January also showed good activity.

Several cyanobacteria and moss samples from the south-western United States were checked for activity for comparison (Table I). Some moss samples were found to have ice-pitting activity and others, even of the same species, were inactive. The active samples were found in winter or at higher elevations where freezing conditions were possible, while the inactive samples were collected in areas that were not subject to freezing. The cyanobacterial samples were all collected from relatively warm areas during summer. A trace of activity was found in a dry soil sample from southern Utah and the others showed no activity.

Discussion

The present results show that macromolecular ice-active substances are associated with a wide range of terrestrial and freshwater photosynthetic organisms in the McMurdo Sound region of Antarctica. These molecules bind to ice crystals, as shown by both their effect on the morphology of growing ice (Fig. 1) and by their incorporation in growing ice (Fig. 3). All

https://doi.org/10.1017/S0954102000000493 Published online by Cambridge University Press

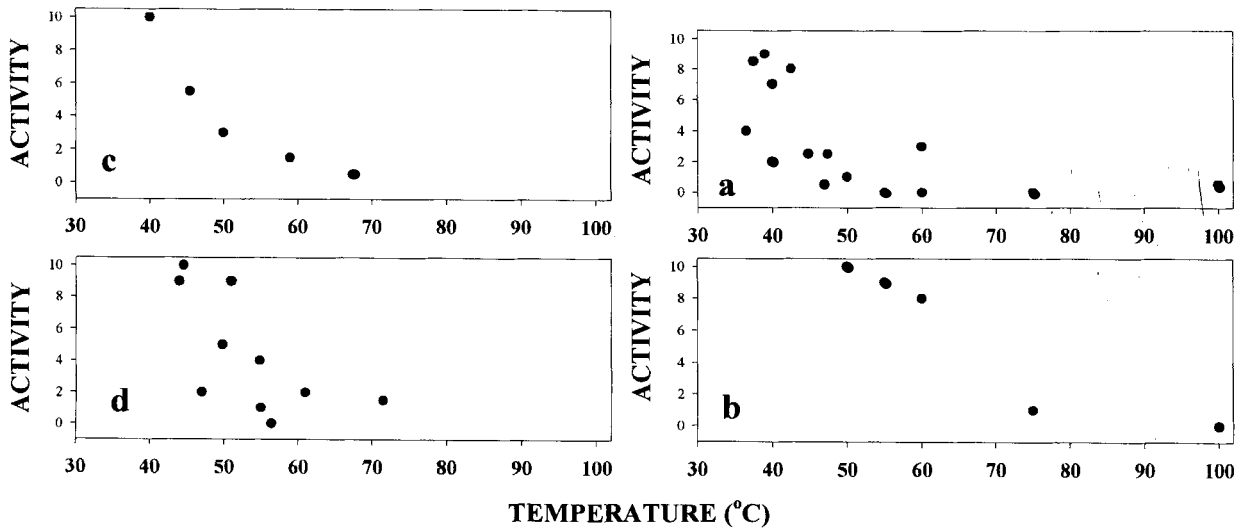

Fig. 4. Thermal stability of ice-active substances. Points show ice-pitting activity of individual extracts of algal and plant tissues after incubation at the indicated temperature for 5 min. **a.** Cyanobacterial mat containing mostly *Phormidium*, **b.** cyanobacterial mat containing mostly *Nostoc*, **c.** *Prasiola* sp., **d.** *Bryum* sp. (moss).

of the *Prasiola* and moss samples examined were ice active, whereas the activities of the cyanobacterial samples, especially the light-coloured mats, were more variable. The reason for this variability is not known, but it may be due to the different freeze-thaw histories of these samples. The Cape Evans mat samples, which were relatively dry samples collected from the edges of small, receding ponds, were among the most active mat samples. Thus, it is possible that their recent exposure to freezing conditions could have increased their activities. Some preliminary data suggest that exposing wet, low activity samples to cold, dry Antarctic weather for one to two weeks can increase activity, but further experiments will be needed to confirm this.

Whereas many of the Antarctic cyanobacteria samples showed ice activity, none of the cyanobacteria from relatively warm environments showed activity. Furthermore, several North American mosses showed activity roughly in proportion to their exposure to cold environments (Table I). These results suggest that the cyanobacterial and moss IASs are generated in response to cold environments, and are not necessarily restricted to polar environments. However, we can not presently rule out the possibility that the IASs are produced by

Table I. Ice-pitting activities of cyanobacteria and moss samples from the south-western United States.

Species	Location+	Date of collection	Snow present?	Elevation (m)	Activity
Mosses					
Syntrichia caninervis#	RR	*c.*15 Feb 1998	–	2000	8
Syntrichia caninervis#	SM	*c.*15 Sep 1998	–	760	trace
Syntrichia caninervis #	SM	*c.*1 Oct 1998	–	1250	0
Syntrichia caninervis#	SM	*c.*15 Oct 1998	–	1800	0
Syntrichia caninervis	LM	6 Apr 2000	–	700	0
Syntrichia norvegica	MC	22 Mar 1999	+	2350	6
Syntrichia sp.	RR	12 Dec 1999	–	1200	0
Bryum sp.	MC	22 Mar 1999	+	2350	8
Grimmia anodon	MC	22 Mar 1999	–	1500	1
Grimmia sp.	LM	6 Apr 2000	–	700	0
Didymodon tophaceus##	MV	18 Aug 1999	–	1140	trace
Cyanobacteria					
Anabaena sp.	MV	23 Mar 1999	–	1140	0
soil cyanobacteria**	CL	25 Aug 1999	–	1350	2
Nostoc spp.*#	NM	15 Jul 1999	–	1350	0

= stored at room temperature in dried state 6–9 months ## = stored at 4°C in semihydrated state 9 months

+RR = Red Rock Canyon, NV, CL = Canyonlands National Park, UT, MV = Meadow Valley Wash, Caliente, NV, MC = Mount Charleston, NV, SM = Lucky Strike Canyon, Spring Mountains, NV, LM = Lake Mead National Recreation Area, NV, NM = Sevilleta National Wildlife Refuge, NM.

*4 samples, all tentatively identified as *Nostoc* spp., assayed separately

**most common cyanobacteria in soil in this area are *Microcoleus, Nostoc and Scytonema* (T. Troxler, USGS, personal communication 1999).

https://doi.org/10.1017/S0954102000000493 Published online by Cambridge University Press

microorganisms that are associated with the algae and mosses. Further studies, such as immunohistochemical studies, will be needed to answer this question. Regardless of the source of the IASs, however, they may have a role in the ability of the algae and mosses to withstand freeze-thaw cycles, which commonly occur during the summer in Antarctica and in the early spring in temperate regions. It should be noted that the ice pitting activity observed here may not have any functional significance in itself, as it may be only a manifestation of the ice binding properties of the IASs. However, these pits formed in the same way in which pits form in the presence of fish antifreezes (Raymond *et al.* 1989) and sea ice diatom IASs (Raymond *et al.* 1994). Unlike the fish antifreezes, however, the IASs examined in this study did not show any freezing point depressing activity, i.e. they did not depress the freezing point below the freezing point expected on the basis of the colligative properties of the solution.

Several studies have reported an increased cold hardiness in the polar organisms investigated in this study. Antarctic cyanobacteria tended to be more tolerant of freezing and thawing than cyanobacteria from Wisconsin (Holm-Hansen 1963), *Prasiola crispa* (Lightfoot) Menegh. has been shown to have a high tolerance to desiccation and freeze-thaw cycles (Davey 1989, Becker 1982, Jacob *et al.* 1992, Jackson & Seppelt 1995), and Arctic mosses were found to withstand much lower temperatures without freezing injury than could temperate zone mosses (table 6-1 in Sakai & Larcher 1987).

The IASs may have a role in the cold hardiness of these organisms, but it is likely that several complementary mechanisms are involved. For example, *Prasiola* undergoes a number of changes during cold acclimation, including accumulation of sugar phosphates which may protect against freezing (Becker 1982), proline (about 30 uMol g^{-1} dry weight) which may stabilize membranes (Jackson & Seppelt 1995) and inorganic phosphates (at the expense of polyphosphates), which may be related to desiccation tolerance (Bock *et al.* 1996). The desiccation tolerance of *Prasiola* may also be due to strong cell walls (Jacob *et al.* 1992). A modest increase in polyol content was observed in one Antarctic moss in winter (Tearle 1987), and a club moss collected in the Midwestern United States in winter was found to have a thermal hysteresis protein (Duman & Olsen 1993). The IASs investigated here do not exhibit thermal hysteresis, so they appear to be a different type of molecule.

Several molecules from bacteria and plants from non-polar environments have been shown to have various effects on ice. Bacterial molecules include ice-nucleating proteins (Wolber & Warren 1989), thermal hysteresis proteins (Duman & Olsen 1993) and an ice-nucleating protein from a cold adapted strain of *Pseudomonas* that modified the habit of growing ice crystals (Xu *et al.* 1998). Plant molecules that have an effect on ice include several proteins from cold-acclimated plants that modify the habit of growing ice (Yu & Griffith 1999, Wisniewski *et al.* 1999), inhibit recrystallization (Yu & Griffith 1999, Worrall *et al.* 1998), and have a thermal hysteresis property (Urrutia *et al.* 1992, Duman 1993, Wisniewski *et al.* 1999). In addition, a number of plant cold response (COR) genes have been identified in *Arabidopsis* that, when coordinately induced, have been shown to increase its freezing tolerance (Jaglo-Ottosen *et al.* 1998). It should be noted that polysaccharides may also have a role in freezing resistance, e.g. in strengthening cell walls, as has been proposed for barley (Olien & Smith 1981). However, to our knowledge, there are no reports of polysaccharides that retard growth on specific faces of ice crystals, possibly because well-defined configurations such as those found in proteins are required for ice binding.

Freezing injury is thought to be due to either physical disruption of cells by extracellular ice, or more commonly, to severe dehydration of the cells as a result of the growth of extracellular ice (Thomashow 1998). The latter can result in denaturation of proteins, precipitation of molecules and membrane damage. Rapid thawing can also be injurious to cells due to the strong osmotic stresses that it creates. The functions of the ice-active proteins that have previously been reported in plants are not clear, but at least some of them are thought to stabilize membranes against freeze-induced damage (Thomashow 1998).

Because the dehydration process that occurs during freezing is in many ways similar to the related problem of desiccation, it is worth mentioning the extraordinary ability of some cyanobacteria, notably the cosmopolitan *Nostoc commune* Vaucher, to withstand long periods of desiccation (Potts 1999). Under normal conditions, this species produces large amounts of extracellular glycan, which accounts for much of the weight of dried *Nostoc* colonies. The purpose of the glycan is not clear, but it may act as a repository for water (Hill *et al.* 1994) or have a role in preventing fusion of membrane vesicles during desiccation (Potts 1999). Embedded in the glycan is a group of proteins, called water stress proteins because they are synthesized during desiccation. The function of these proteins is not clear, but they may be involved in modifying the structure of the glycan (Potts 1999). Further studies will be needed to determine whether such glycan-protein complexes are related to the cyanobacterial ice-active substance described here.

The present results provide only limited information on the chemical nature of the IASs. They are large molecules, perhaps with molecular weights exceeding 300 kDa, but if they have extended rather than globular configurations, their sizes may be smaller than this. The IASs appear to have a protein component because their activity is destroyed at temperatures in the range 45–65°C, a range in which many proteins are denatured (Privalov 1979). However, the possibility that some non-protein part of the molecules is denatured at these temperatures can not be presently ruled out. In these respects, as well as in their ice-pitting and ice-binding properties, the IAS molecules appear very similar to IAS molecules associated with sea ice diatoms from McMurdo Sound (Raymond *et al.* 1994). Recent results indicate that the

latter molecules are glycoproteins (Raymond 2000). Presently we are attempting to identify the chemical nature of the cyanobacterial, algal and moss IASs, using their ice-binding properties to purify them. Initial results with the cyanobacterial IAS indicates that it is also a glycoprotein.

Acknowledgements

We thank Dr Lloyd Stark, UNLV, for valuable assistance in identifying and providing moss samples; Dr Lynda Goff, University of California, Santa Cruz for identifying several algal species; Dr Jay Jones and Brian Harper, UNLV, for providing moss and cyanobacterial samples from Meadow Valley Wash, NV; Dr Anne Cross, Oklahoma State University, for providing cyanobacteria samples from New Mexico, Dr Jayne Belnap, US Geological Survey, for providing a cyanobacterial sample from Utah; Drs John Priscu and Edward Adams, Montana State University, and Dr David Ainley for assisting in the field collections, and Ms Julie Seidlinger for valuable laboratory and field assistance. We thank the referees, G. Warren and M. Potts for their helpful comments. This study was supported by NSF grants OPP-9814972 to CHF and OPP-9814294 to JAR.

References

Becker, E.W. 1982. Studies on Antarctic *Prasiola crispa* and *Nostoc commune* at low temperatures. *Polar Biology*, **1**, 99–104.

Bock, C., Jacob, A., Kirst, G.O., Liebfritz, D. & Mayer, A. 1996. Metabolic changes of the Antarctic green alga *Prasiola crispa* subjected to water stress investigated by in vivo 31P NMR. *Journal of Experimental Botany*, **47**, 241–249.

Broady, P.A. 1996. Diversity, distribution and dispersal of Antarctic terrestrial algae. *Biodiversity and Conservation*, **5**, 1307–1335.

Buckley, H.E. 1952. *Crystal growth*. New York: Wiley, 339 pp.

Cheng, C.C. & DeVries, A.L. 1991. The role of antifreeze glycopeptides and peptides in the freezing avoidance of cold-water fish. *In* di Prisco, G., *ed. Life under extreme conditions*. Berlin: Springer Verlag, 1–14.

Davey, M.C. 1989. Effects of freezing and desiccation on photosynthesis and survival of terrestrial Antarctic algae and cyanobacteria. *Polar Biology*, **10**, 29–36.

Duman, J.G. 1993. Purification and characterization of a thermal hysteresis protein from a plant, the bittersweet nightshade *Solanum dulcamara*. *Biochimica Biophysica Acta*, **1206**, 129–135.

Duman, J.G. & Olsen, T.M. 1993. Thermal hysteresis protein activity in bacteria, fungi and phylogenetically diverse plants. *Cryobiology*, **30**, 322–328.

Goldman, C.R., Mason, D.T. & Wood, B.J. 1972. Comparative study of the limnology of two small lakes on Ross Island, Antarctica. *Antarctic Research Series*, **20**, 1–50.

Hill, D.R., Peat, A. & Potts, M. 1994. Biochemistry and structure of the glycan secreted by desiccation-tolerant *Nostoc commune* (Cyanobacteria). *Protoplasma*, **182**, 126–148.

Jackson, A.E. & Seppelt, R.D. 1995. The accumulation of proline and *Prasiola crispa* during winter in Antarctica. *Physiologia Plantarum*, **94**, 25–30.

Jacob, A., Weincke, C., Lehmann, H. & Kirst, G.O. 1992. Physiology and ultrastructure of desiccation in the green alga *Prasiola crispa* from Antarctica. *Botanica Marina*, **35**, 297–303.

Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O. & Thomashow, M.F. 1998. *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science*, **280**, 104–106.

Hawes, I., Smith, R., Howard-Williams, C. & Schwarz, A.-M. 1999. Envirionmental conditions during freezing, and the response of microbial mats in ponds of the McMurdo Ice Shelf, Antarctica. *Antarctic Science*, **11**, 198–208.

Holm-Hansen, O. 1963. Viability of blue-green algae after freezing. *Physiologia Plantarum*, **16**, 530–540.

Lamb, I.M. 1970. Antarctic terrestrial plants and their ecology. *In* Holdgate, M.W., *ed. Antarctic ecology*, Vol. 2. London: Academic Press, 733–751.

Lovelock, C.E., Jackson, A.E., Melick, D.R. & Seppelt, R.D. 1995. Reversible photoinhibition in Antarctic moss during freezing and thawing. *Plant Physiology*, **109**, 955–961.

McKnight, D.M., Alger, A., Tate, C.M., Shupe, G. & Spaulding, S. 1998. Longitudinal patterns in algal abundance and species distributions in meltwater streams in Taylor Valley, southern Victoria Land, Antarctica. *Antarctic Research Series*, **72**, 109–127.

Olien, C.R. & Smith, M.N. 1981. Protective systems that have evolved in plants. *In* Olien, C.R. & Smith, M.N., *eds. Analysis and improvement of plant cold hardiness*. Boca Raton: CRC Press, 61–87.

Potts, M. 1999. Mechanism of desiccation tolerance in cyanobacteria. *European Journal of Phycology*, **34**, 319–328.

Privalov, P.L. 1979. Stability of proteins. Small globular proteins. *Advances in Protein Chemistry*, **33**, 167–241

Raymond, J.A. 2000. Distribution and partial characterization of an ice-active molecule associated with sea ice diatoms. *Polar Biology*, **23**, 721–729.

Raymond, J.A. Wilson, P. & DeVries, A.L. 1989. Inhibition of growth of non-basal planes in ice by fish antifreezes. *Proceedings of the National Academy of Sciences, USA*, **86**, 881–885.

Raymond, J.A., Sullivan, C.W. & DeVries, A.L. 1994. Release of an ice-active substance by Antarctic sea ice diatoms. *Polar Biology*, **14**, 71–75

Sakai, A. & Larcher, W. 1987. *Frost survival of plants. Responses and adaptation to freezing stress*. Berlin: Springer Verlag, 321 pp.

Tearle, P.V. 1987. Cryptogamic carbohydrate release and microbial response during spring freeze-thaw cycles in Antarctic fellfield fines. *Soil Biology and Biochemistry*, **19**, 381–390.

Thomashow, M.F. 1998. Role of cold-responsive genes in plant freezing tolerance. *Plant Physiology*, **118**, 1–7.

Urrutia, M.E., Duman, J.G. & Knight, C.A. 1992. Plant thermal hysteresis proteins. *Biochimica et Biophysica Acta*, **1121**, 199–206.

Wisniewski, M., Webb, R., Balsamo, R., Close, T.J., Yu, X.-M. & Griffith, M. 1999. Purification, immunolocalization, cryoprotective and antifreeze activity of PCA60: a dehydrin from peach (*Prunus persica*). *Physiologia Plantarum*, **105**, 600–608.

Wolber, P. & Warren, G. 1989. Bacterial ice-nucleation proteins. *Trends in Biochemical Science*, **14**, 179–182.

Worrall, D., Elias, L., Ashford, D., Smallwood, M., Sidebottom, C., Lillford, P., Telford, J., Holt, C. & Bowles, D. 1998. A carrot leucine-rich-repeat protein that inhibits ice recrystallization. *Science*, **282**, 115–117.

Xu, H., Griffith, M., Patten, C.L. & Glick, B.R. 1998. Isolation and characterization of an antifreeze protein with ice nucleation activity from the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Canadian Journal of Microbiology*, **44**, 64–73.

Yu, X.-M. & Griffiths, M. 1999. Antifreeze proteins in winter rye form oligomeric complexes. *Plant Physiology*, **119**, 1361–1369.