The Role of Bacterial Protein for Snowmaking and as an Antifreeze

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Abstract--Since making of artificial snow for ski area application is now widely practiced at most major resorts, an understanding of the basis for the process is desirable for ski professionals. An outline of the supercooling of liquid water and the ordering transition necessary to form the crystalline state will be presented. Natural and inorganic nucleating agents do the job but rather inefficiently. Depending on water droplet size natural agents will nucleate ice at 18°F on the average, while bacterial proteins are effective at 25°F to 28°F. Thus snow can be made more efficiently and the liquid water content may be controlled for the application. This paper will address some the engineering as well as environmental concerns of snow making. Related to this discussion is the prevention of nucleation and growth of ice in biological fluids or plant structures. A brief survey will show how similar proteins prevent ice crystal growth in living tissue as well as the application for preservation of frozen food products.

Introduction

This paper will present a brief introduction to the formation of snow in the atmosphere to emphasize the physical conditions that must be present for the process to occur. Because water is a substance of exceptional properties, a short outline of the phase transitions between vapor, liquid and solid forms is needed to appreciate the phenomena, not only the formation of snow but the metamorphism that occurs in the snowpack with time. There is a vast scientific literature dealing with this topic; only the bare outline of the relevant factors will be presented.

Skiers and ski area operators are concerned with the natural formation of snow; the siting of ski area facilities is dictated by the snowfall distributions on mountain ranges. Snow accumulation zones from precipitation and wind transport play a large role in the avalanche hazard factors in all types of skiing. In recent years the incorporation of artificial snow making into commercial ski operations is almost universally practiced world wide. Cloud seeding for snow production continues to be practiced, but it is not nearly so wide spread nor as effective as the local area production. The economic feasibility of the process has been largely dependent on the commercial development of bacterial protein seeding agents which become effective at temperatures of only about -2°C (28°F). Natural snowfall does not necessarily occur when the weather becomes cool enough nor in sufficient quantity every season to sustain the effective operation at the

modern resort facility; the local production of the necessary snow cover gives the operators assurance of season-long skiable conditions.

I came upon this topic via a back door, the role of proteins as antifreeze agents in cold water fish. The blood plasma of Antarctic fish will freeze at about -1.5°C while the water in which they live is at about -2.5°C. In a similar but more practical avenue the performance of antifrost agents for field crops came to be studied. There are now proposals to use nucleating agents for creation of artificial ice for refrigeration in the manner of the earlier lake ice storage used for rail refrigeration. It turns out to be economically feasible to create masses of ice using atmospheric cooling for freezing and then storing for year around use rather than operating a mechanical heat pump (refrigerator). On the one hand antifreeze proteins prevent ice crystal growth and on the other similar proteins serve as very effective nucleating agents.

To understand the formation of clouds, which are supercooled liquid water, and snow in the atmosphere refer to Figure 1 which shows a part of the phase diagram for water. Pressure is plotted as the ordinate and temperature as the abscissa; the volume for a given mass is not shown but changes with temperature, pressure and the change in phase. Ice I is the variety of solid water that occurs in nature. At sufficiently high temperature assume all the water is in the vapor state i.e. at some point A in the area marked vapor. Upon cooling at constant pressure the state point moves to the left until it contacts the line separating vapor and liquid. At that point vapor may condense to form liquid as for example cloud droplets in the atmosphere. If the vapor-liquid mass is cooled further, the temperature does not change until all the vapor is condensed to liquid because the heat of vaporization must be liberated and keeps the temperature constant. If only liquid is present it may be cooled until the freezing temperature represented by the ice-liquid phase line is reached. At this temperature, given appropriate conditions, the liquid will freeze to form ice with the temperature remaining constant because heat of fusion is liberated. When all the liquid is frozen, the ice may be further cooled to point B. Below the triple point the vapor may condense to the solid state (frost) directly, and the solid may vaporize or sublime.

Water does not, without cause, change it's phase state at the at the phase boundary conditions shown in the diagram, so the lines demarking phase change are very fuzzy. Liquid water as in cloud droplets can be cooled to about -40°C before it will certainly freeze. To freeze at higher temperatures a nucleating site to assist the molecules to arrange themselves in the correct structural pattern must be present. Even vapor will not condense without a foreign particle upon which to form the droplet. Most atmospheric clouds are in fact liquid to a temperature of about

-20°C. Some supercooled droplets may freeze in the atmosphere, and once formed into ice crystals, condense vapor to grow at the expense of the liquid cloud droplets because the vapor pressure over the liquid is higher than that over the ice. See the dashed line below the triple point. Thus in a snow storm there are vapor, liquid cloud droplets and snow flakes simultaneously. The storm may deposit rime or liquid droplets freezing on contact with a cold surface and snow at the same time.

The process of cloud seeding in nature is done over the ocean by the presence of sea salt particulates. Over land it is the result of clay or mineral particles upon which the water condenses. Every single cloud droplet has a foreign particle within it; the droplet would not form without such a particle. A very small droplet or ice crystal will evaporate rather than grow. Thus large droplets of ice crystals grow at the expense of small ones. This discussion indicates in a qualitative manner how any condensation is formed at all.

Artificial seeding agents are used to initiate snow formation in the atmosphere. The most successful have been silver iodide or cupric sulphide until the introduction of proteins derived from the membrane structure of certain bacteria. This paper will discuss the process in the context of artificial snow making only. For a broad overview of the problem refer to the book by Lock (1) or a review of bacterial nucleating agents by Wolber (2).

The Process for Artificial Snow Making

The physical and engineering requirements for artificial snow making have been known for some time. The basic process involves mixing cold water with air emerging from a high pressure nozzle; the water emerges as a cloud of droplets having diameters set by the gun conditions but in the range of 100 to 700 micron diameter. 100 microns = 0.004 in. The adiabatic expansion, that is expansion where the work done by the gas mass during expansion comes from the gas itself, cools the nozzle air substantially, but the cooling needed to freeze the water injected into the atmosphere must come from the surrounding atmospheric air. The engineering details are described by Chen and Kevorkian (3).

All natural water has foreign particles which serve as the nucleating agents, but the average freezing temperature is about -8°C or 18°F. Figure 2 taken from the marketing literature of Snowmax Technologies (4) shows data for water samples from a variety of ski areas. Thus no frozen droplets would be produced unless the ambient air temperature were below that temperature. Cooling of the warm droplets occurs by conduction from the droplet to the air and by evaporation until a wet bulb temperature depending on the relative humidity of the atmosphere is reached. Then droplet freezing must occur before it falls to the ground or evaporates. Most likely the droplets will fall to the ground as

liquid and then be nucleated immediately by ice present to form a slab of solid ice. If it were seeded, the time to cool and freeze must be less than the hang time in the air. Droplets in the range of 200 to 700 microns will hang for about 15 seconds which is sufficient to freeze completely. Droplets with some residual liquid will yield a solid ice mound on the ground. Note this is quite different from atmospheric snow formation where the hang time is large and the grains grow by vapor accretion depending on the relative humidity or the degree of supersaturation of the water vapor in the air relative to the snow grain. Artificial snow will be denser in general than natural snow, and it is apt to have more liquid and hence freeze to a solid mass. Yet the higher density makes it desirable as the base course for groomed ski slopes.

Figure 3 shows four views of a micro-crystallite ice model (11) showing the crystal structure. The grain shown is part of prism with steps on the prism faces. View (a) shows the oxygen atom arrangement looking along the c or hexagonal symmetry axis; view (b) looks parallel to the basal plane or at right angles to the symmetry axis and along the prism face, and is a right elevation view. View (c) on the left is the elevation view looking parallel to a prism face, and view (d) is the front elevation looking normal to a prim face. Only the oxygen atoms are shown. The bond links between each oxygen atom contain one proton or hydrogen ion and two pairs of bond electrons. Each oxygen has four possible bonds. Notice that on plane surfaces there are oxygens lacking just one bond, but at convex corners there are oxygens that may lack two bonds. A water molecule may link up to any of the incompleted bonds on the ice crystal; at the same time any water molecule attached to the faces or edges of the crystal may be shaken loose by thermal vibration. This crystal model illustrates the fact that as the radius of curvature of the exterior of an ice grain decreases the vapor pressure around the grain increases because water molecules may be broken free easier from the convex corners. For the same reason if the ice grain is immersed in liquid, the melting temperature of a grain of small external radius will be depressed from that given by the phase diagram as shown in Figure 1. The model shows by the stubs the favorable bonding sites at the junction of the basal and prism faces. Wherever an oxygen does not have it's four completed bonds is a bonding site.

The process of evaporation or melting of an ice grain depends then not only on the temperature and pressure but on the curvature of the grain as well. For the ice grain to accrete water molecules the process of only cooling does not insure that the long molecular polymers in the liquid state will necessarily line up as required by the hexagonal structure of the solid. The liquid water molecule has the property that, because the oxygen has four bond sites two of which are occupied by hydrogen, it prefers near the freezing temperature to link up in a somewhat disordered manner with other molecules to form long chain polymers. One of the reasons for the large supercooling of water is that the polymer strings in the

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liquid may be 10 water molecules or more in length. Hence one may appreciate the necessity for a template to which the polymers may attach and arrange their structure to become that of the ice crystal structure. It is not surprising that liquid water can be cooled to -40°C before it changes to crystalline solid rather than becoming a glass-like solid. These properties result in part from the fact that water ice expands relative to that of the liquid by 8%.

Figure 4 serves to summarize the statements above regarding the melting and freezing of water between the liquid and solid phases. The ice point temperature might be taken as 0°C or at the triple point. If a large piece of ice is immersed in liquid water, that is the temperature observed for the system. The stable equilibrium freezing temperature T^i may be depressed by pressure, curvature of the boundary or by solutes in the liquid. Below T^i the liquid is supercooled or metastable. Nucleation is necessary for the solid to form and grow. At T^* spontaneous freezing will occur.

Microbial Ice Nucleation

It has been observed for some time that biological structures provide a lattice matching template for the formation of ice in nature. Lichens provide such material, but the most frequently observed material comes from several natural bacterial organisms. See Wolber (2) or Warren and Wolber (5) for details. For these materials the ice nucleating activity operates between -10°C and -2°C. To operate at such high temperature the ice embryo must be relatively large to align the liquid polymers. They must have a hexagonal structure and bond spacing which closely matches that of the ice. There is much interest in the properties of the bacterial Ina proteins, a single one of which, is present in each bacterial strain. For the purposes of commercial snow making the material developed by Snowmax Technologies is produced by the bacterium Pseudomonas syringae. This bacterium occurs widely in nature.

The protein present in the cell membrane is the active agent and varies in composition. Figure 5 taken from Warren and Wolber (2) shows how the protein makes up the cell membrane. The protein is composed of amino acid chains of 8 units coupled to form 16 member chains of which 3 are coupled to a 48 unit structure. The 48 unit structures are repeated with the fidelity increasing towards one end. It is the high fidelity of the chains coupled with the helical protein structure that makes them good templates for ice. Note that the 48 unit structure is composed of 6 of the 8 unit building blocks which coil to hexagonal structures. These units associate in the cell membrane to form the hexagonal templates for nucleation. The high fidelity and the large association complex yields the high nucleation temperature. The molecular weight of templates for nucleation temperatures above -3°C is 19000 kiloDalton. 16 Daltons is the mass of one oxygen

atom. The bond length between carbon or nitrogen atoms in the protein is comparable to that between oxygens in ice, so these structures are of the order of 100 unit cell lengths of the ice lattice. Figure 5 shows the membrane and the hexagonal template domains on the membrane surface.

The mechanism of these template sites for alignment of water molecules or water polymers is not well known. What must occur, however, are molecular bonds which hold the water molecules in appropriate geometric array so that further accretion may occur to permit the embryo to grow to a full ice crystallite to which water molecules will accrete directly.

Technical and Environmental Concerns

In practice the nucleating agent is fed into the supply water of the snow gun in quantity sufficient to insure a good probability for freezing of the 200 to 700 micron droplets so that little if any liquid remains by the time they settle to the ground. The high threshold temperature for freezing permits longer freeze time and better heat transport of the heat of fusion out of the droplet. The bacterium strain P. syringae originally isolated from a corn plant is cultivated and then sacrificed by freeze drying so that the protein mass contains no live cells. Furthermore this bacterium is widely distributed in the environment and poses no environmental or health threat in its use. Figure 6 from Rice (6) shows the nucleating efficiency of the material in atomized water as a function of the ambient air temperature. All natural water contains some nucleation particulates, so there are always some droplets which become frozen depending on the supercooling of the liquid. With the protein material in the water every droplet freezes below 27°F.

A study done at Courchevel in 1991 showed that with an average temperature of -14°C the ratio of snow volume to water volume used was 3.15 with Snomax and 1.90 without. The snow density was 400 to 440 kg/m^3 with and 500 to 520 kg/m^3 without. Liquid water content was small in either case. The snow crystal shape obtained without Snomax was rounded of 0.5 to 1 mm in diameter. With Snomax the grains were much smaller and varied in shape. However, probably the overriding factor for the use of a nucleating agent is the higher operating temperature at -2° to -3°C instead of -10° to -12°C. One could not make snow otherwise.

Studies were also made on the after-effects of the use of Snowmax at the Calgary Winter Olympics in 1988. From data taken on snow and soil samples there was no build up of environmental ice nucleation activity. No visible damage from frost or other sources was noted nor change in plant species observed. P. syringae was not isolated in any of the field samples.

Glycoproteins as Antifreeze Agents

Closely related to the development and operation of ice nucleating agents (Ina) is the development and performance of antifreeze proteins. Papers by Feeney and Yeh (7), DeVries (8) and Eastman and DeVries (9) give background and references for this work. Only a brief outline will be given here. These proteins first were noted as the agent in the blood plasma of arctic fish which prevented freezing. The attention of food technologists was drawn to them as agents to preserve the quality of frozen foods by prevention of ice crystal growth in the food. There is also interest in their use as agents to prevent frost damage in the agriculture of fruits and vegetables.

A substantial body of evidence of these agents shows that they are proteins quite similar to the ice nucleating materials. The antifreeze proteins have a helical structure, but usually have disaccharide appendages attached to the basic helix. The disaccharide structure is a linear array of two simple sugar rings in tandem. The backbone helix is largely hydrophobic in character; that is it does not bond to water molecules. The disaccharide structure is hydrophilic, bonding readily with water. The protein is labelled antifreeze glycoprotein (AFGP). The spacing of the hydrophilic sites on the disaccharides matches closely the spacing of the available bond sites on the prism faces of the ice crystal. See Figure 3.

Studies of ice crystal growth show a stepped structure as shown in Figure 7 taken from DeVries (8). The basal plane, prism faces and the steps that normally develop because of the preferential accretion of water are shown. Growth of the hexagonal ice is primarily on the basal planes along the direction of the a-axes. Figure 8, also from DeVries (8) shows what is believed to be the mechanism by which the AFGP structures inhibit the growth. The hydrophilic bond sites permit the protein to attach to the embryo ice crystal along the steps. In the ice crystal model shown in Figure 3 long and short stubs are placed along the concave junction of the basal and prism face planes. The long stubs represent a proton bonded to the oxygen; the short stubs represent only a bond site without an hydrogen atom. The multitude of bond sites along this junction suggests why the disaccharides of the antifreeze protein are more likely to bond there. Water molecules may then accrete between the AFGP aggregates but not to them since they are hydrophobic except at the disaccharide clusters. The ice crystal surface grows where possible but now with surfaces with a small radius of curvature or high curvature. Remember that the freezing point is depressed by highly curved surfaces, so the further growth is impeded unless the temperature is sufficiently lowered. At the constant temperature of the fish body fluid growth ceases. The encapsulated ice embryo is treated like other cellular structures in the fish plasma. Only if the ice crystal grows to sufficient

size will the circulation of body fluids be impeded. The external water temperature in the arctic ocean may be as low as -2.5°C, but the body fluid circulation remains.

For the application to frozen food preservation prevention of large ice crystal growth in the foodstuff is desirable. Glycoproteins will serve that purpose. In agricultural crops similar prevention of growth of large ice crystals will protect the plant structure from damage by ice growth.

This is a very short outline of the study of ice formation and nucleation by bacterial proteins. There is a large published literature on the microbiological aspects of the problems. Nevertheless since the application to ski area operations has become so important, it is hoped that snow science personnel would appreciate and benefit from this discussion. It was of interest to me after writing this paper to read in the science section of The Economist (10) of the great interest by biotechnologists in the applications of glycoproteins for all kinds of wonder-drugs for immunological disease.

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3.   Chen, J.& Kevorkian, V., Heat and Mass Transfer in Making Artificial Snow, Ind. Eng. Chem. Process Des. Develop., Vol 10, Vol. 10 p. 75-78 (1971).

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6   Rice, A.F., Biotechnology-Enhanced Natural Cold Thermal Storage, Presented at 14th World Energy Engineering Congress, 25 Oct. 1991.

7   Feeney, R.E.& Yeh, Y., Antifreeze Proteins: Properties, Mechanism of Action, and Possible Applications, Food Technology-January 1993.

8   DeVries, A.L., Role of Glycopeptides and Peptides in Inhibition of Crystallization of Water in Polar Fishes, Phil. Trans. R. Soc. Lond. B304, 575-588 (1984).

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10 Science and Technology, The Economist, Vol. 332 (7882), pg. 87, Sept. 24-30, 1994.

11 Zometool--The 31 Zone Structural System used here is available from Zometool, P.O. Box 7053, Boulder, CO 803204
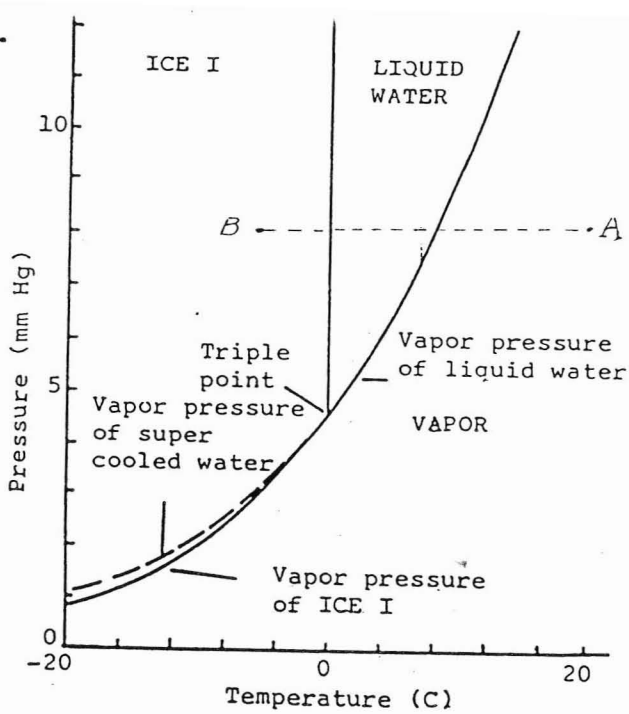
Fig. 1 Phase diagram of water in the region of the triple point where vapor, liquid and solid coexist. The pressure is 4.5 mm Hg and the temperature is 0.0075°C at the triple point.

FREEZING VS. "ADJUSTED" FREEZING TEMPERATURE

WITH SNOMAX
Avg. Freezing Temp. = 26.8 °F.

WITHOUT SNOMAX
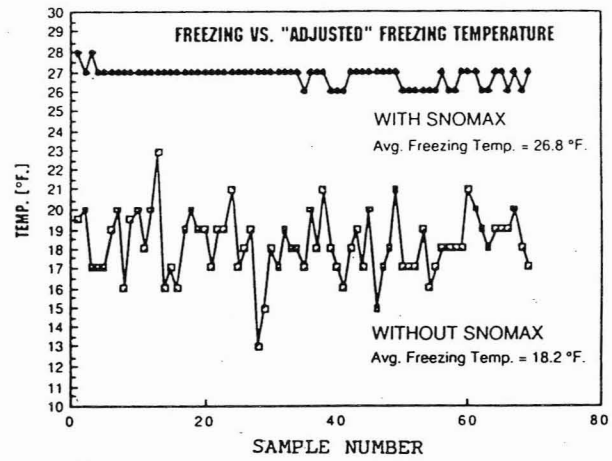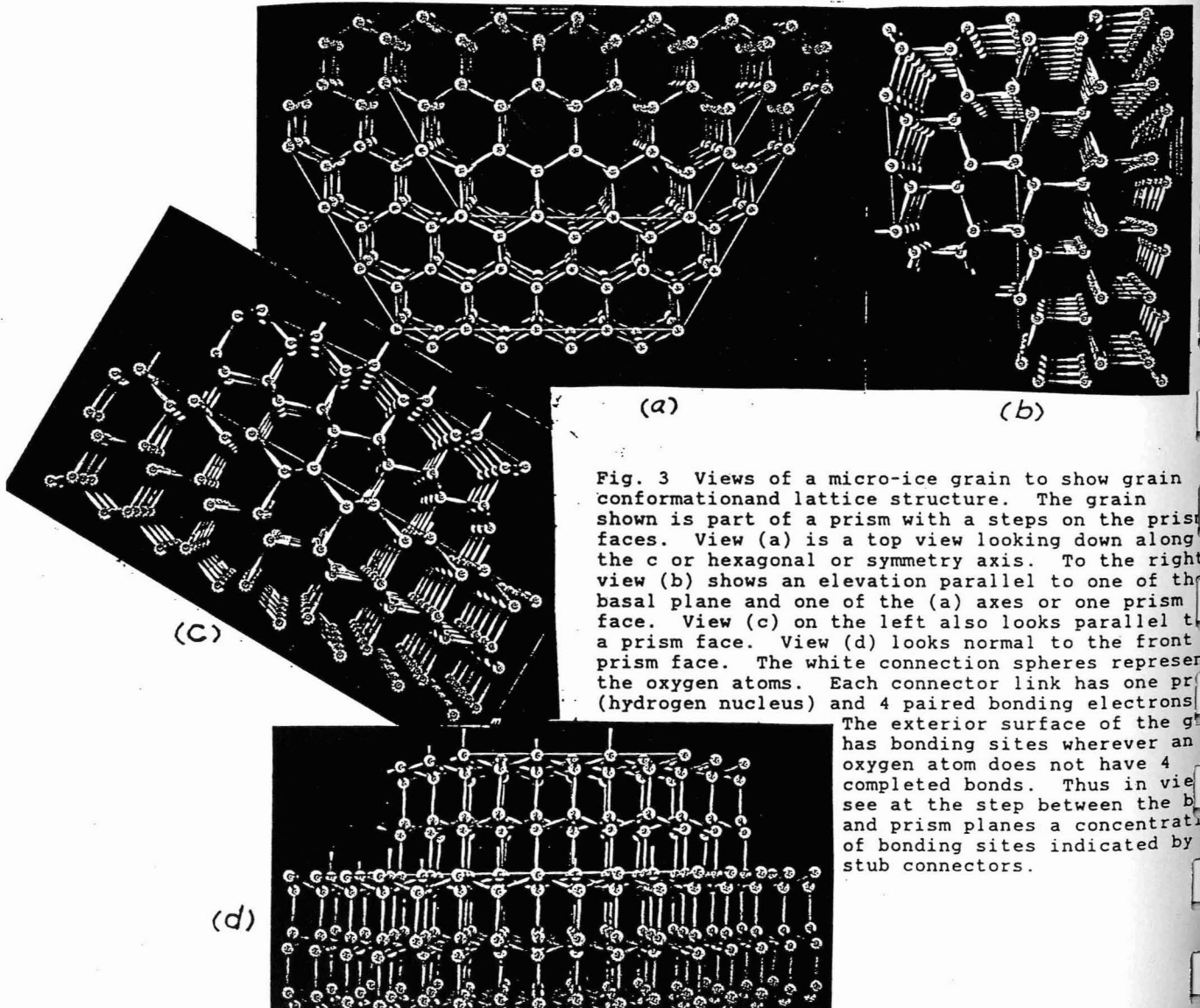Avg. Freezing Temp. = 18.2 °F.

Fig. 2 These data on water sample freezing temperature are provided by Snomax Technologies. Source water samples from ski areas are compared with and without Snowmax. Natural water samples have 0 to 115 ice nucleation sites (INS) per milliliter. With Snowmax the number is 250,000/ml.

(a)

(b)

(c)

(d)

Fig. 3 Views of a micro-ice grain to show grain conformation and lattice structure. The grain shown is part of a prism with a steps on the prism faces. View (a) is a top view looking down along the c or hexagonal or symmetry axis. To the right view (b) shows an elevation parallel to one of the basal plane and one of the (a) axes or one prism face. View (c) on the left also looks parallel to a prism face. View (d) looks normal to the front prism face. The white connection spheres represent the oxygen atoms. Each connector link has one pr (hydrogen nucleus) and 4 paired bonding electrons. The exterior surface of the g has bonding sites wherever an oxygen atom does not have 4 completed bonds. Thus in vie see at the step between the b and prism planes a concentration of bonding sites indicated by th stub connectors.

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| T^o | Freezing temperature (ice point) |

Superheated, <u>stable</u> liquid

Freezing point depression resulting from pressure, curvature, solutes, etc.

| T^i | Stable equilibrium freezing temperature |

Supercooled, <u>metastable</u> liquid

| T^* | Spontaneous freezing temperature |

Fig. 4 Diagram shows the possible freezing point temperature for the liquid to solid transition. The nominal temperature T^o may be modified from the value given in Fig. 1 to stable lower values by the factors shown, and the liquid state remains stable. Below T^i is a supercooled metastable state. A variety of nucleation particles will cause immediate freezing.
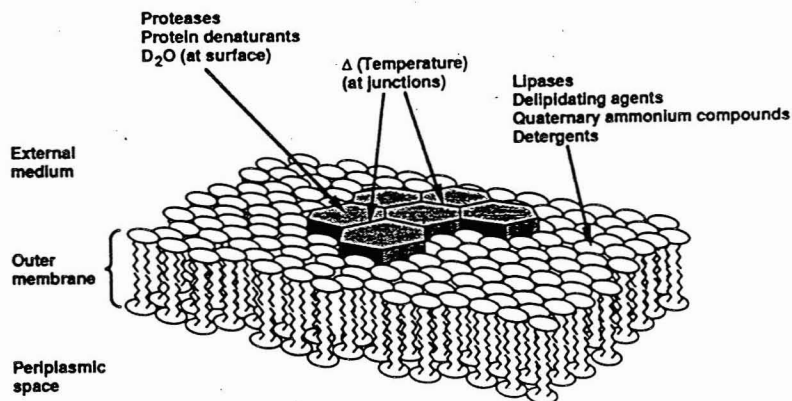

Fig. 5 This figure is taken from Warren and Wolber (2). The structure of the bacterial membrane is shown with a cluster of hexagonally configured and arrayed proteins on the surface. These clusters serve as the nucleation sites for the initiation of ice crystal growth. Shown in the figure are some of the activations agents which destroy the nucleation capacity.

Impact of Ice Nucleation Active Bacteria
Atomized Water in Cold Air

% water droplets frozen

sample size: 83 sources
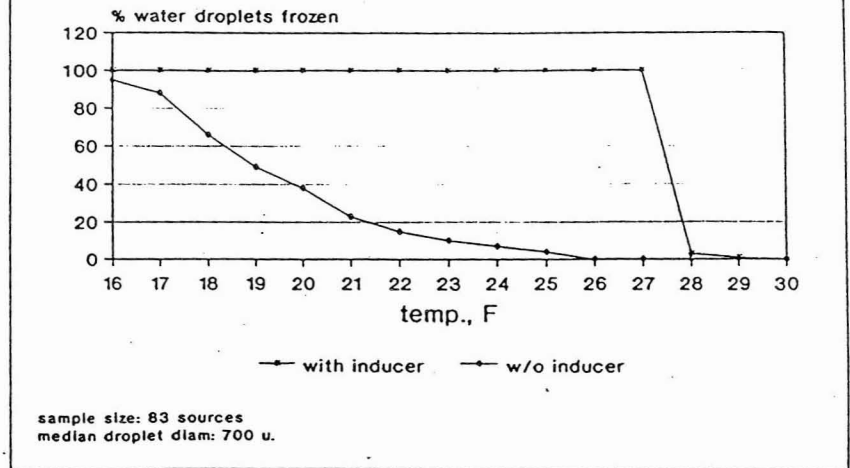median droplet diam: 700 u.

Fig. 6 Figure taken from Rice (5) shows the percent
of liquid droplets which become frozen with and without
nucleation proteins as a function of air temperature
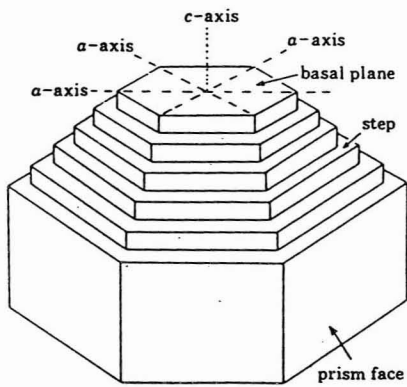at a snow gun.

Fig. 7 Diagram of a hexagonal ice crystal showing
prism faces and steps between basal planes. The
c axis is perpendicular to the basal planes, and the
a axes are parallel to the prism faces. Figure is
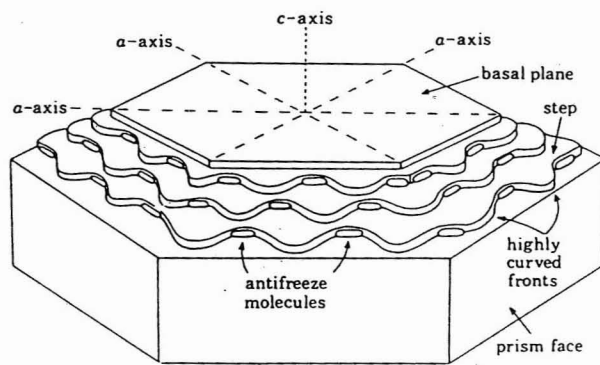from DeVries (7).

Fig. 8 Model shows the bonding sites for antifreeze
proteins at the prism face steps. Subsequent growth
of curved prism face surfaces results in a depression
of the freezing point temperature for these surfaces.
Hence the ice crystal ceases to grow. Figure is taken
from DeVries (7).

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