Toward the Molecular Mechanism of a Commercial Ice Nucleating Agent

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Biology manipulates water crystallization via a variety of protein-mediated mechanisms. To protect organisms against damage induced by ice crystals, ice-binding or antifreeze proteins stick to the outside of existing ice crystals, preventing crystal growth while also inhibiting melting. A protein with such activity, isolated from ocean pout, was commercialized by Unilever for use as an ice structuring food additive for frozen dairy products. Ice-binding proteins are generally small, binding to only a handful of water molecules usually organized into a single row. On the other side of the phase diagram, ice nucleating activity by a protein from Psuedomonas syringae, InaZ, has been demonstrated and also commercialized by Snomax as a snow- or ice-making additive. Recent advances in protein structure prediction by Alphafold have allowed for high-confidence predictive models of InaZ molecular structure, which consists of a >90kDa fiber-like arrangement of beta strand repeats. A recent 2.1A resolution crystal structure of a structurally related bacterial surface layer protein suggests a water binding mode involving two lines of ordered water molecules, facilitating ice crystal nucleation via a clathrate-like mechanism. However, recent work has suggested that biological ice nucleating activity may also rely on oligomeric or higher-order association of ice nucleating proteins. Purified solutions of ice nucleating proteins routinely display lower ice nucleating activity than the commercial agent, Snomax. To probe the molecular mechanism of a known high-efficiency biological ice nucleator, I utilized multi-scale microscopy to analyze the commercial ice nucleator, Snomax. Light microscopy of methylene blue-stained Snomax indicated the presence of particles of several microns in length, consistent with inactivated Pseudomonas syringae. Metal-stained room-temp 120kV transmission electron microscopy confirmed the rod-shaped morphology of the particles and provided an example of rods undergoing septation. Finally, cryo electron microscopy showed the presence of intact organisms consistent with gram-negative Pseudomonas syringae. Overall, these results suggest that the high ice nucleation activity of the Snomax product may be derived from meso-scale structures on the bacterial cell surface, rather than solubilized ice nucleating proteins acting individually. Further structural analysis of high-efficiency ice nucleating agents is required to define the scale of biological structure most suited for ice nucleation.