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Review

Bacterial ice nucleation and its potential application in the food industry

Jingkun Li and Tung-Ching Lee

Extensive investigations of bacterial ice nucleation by strains of *Pseudomonas*, *Erwinia* and *Xanthomonas* have indicated that highly homologous proteins, each encoded by a single gene, are involved in the ice-nucleation active sites of either intact bacterial cells and/or cell-free ice nucleators. The application of these bacterial ice nucleators to the freezing of some model food systems and real foods, such as salmon, egg white protein and cornstarch gels, elevates nucleation temperatures, reduces freezing times and improves the quality (e.g. the flavor and textural properties) of frozen foods; this suggests that there may be profound potential for energy savings and quality improvement in the food industry. The use of bacterial ice nucleators is a unique application of biotechnology, as it directly improves freezing processes. Further research is needed to gain a better understanding of the basic mechanisms, the practical applications and the safety of this particular material in the commercial freezing of food products.

Freezing is one of the best methods available in the food industry for preserving food products with high quality. Many factors affect the economic viability of the freezing process and the quality of the food products. Overall, the efficiency of this process and the resulting food quality are affected by two important factors: supercooling (the cooling of liquid below its freezing point without freezing) and nucleation (the initiation of the crystallization of liquid water into solid ice)[1]. There are potentially two types of ice nucleation. Homogeneous ice nucleation takes place only in extremely purified water, where an ice nucleus is formed by the random accumulation of water molecules. Heterogeneous ice nucleation is predominant in the freezing of real food systems, and occurs when water molecules aggregate in a crystalline arrangement on nucleating agents such as suspended foreign particles, surface films or walls of containers. The type of ice nucleation is determined by the properties of the solutes and the freezing rate, which consequently affect crystal size and crystal structure within the food and, therefore, product quality[2].

Bacterial ice nucleation by strains of *Pseudomonas*, *Erwinia* and *Xanthomonas* has been both acknowledged and investigated since the early 1970s, and it has been recognized as one of the major causes of frost injury in plants[3]. Many studies have contributed to the understanding of ice-nucleation mechanisms[4], and the elimination of ice-nucleation injury in plants[5]. The unique activity of ice nucleation at higher subzero temperatures (in the range –2 to –5°C), however, makes these microorganisms very useful in such processes as the production of artificial snow and the freezing of some food products (Refs 6, 7 and J.M. Ryder, PhD thesis, University of Rhode Island, Kingston, RI, USA, 1987), where ice nucleation is a limiting step.

The aim of this article is to present both basic and up-to-date knowledge of bacterial ice nucleation, and to discuss the application of this fascinating phenotype to the study of freezing foods as well as the potentially profound impact it may have on energy savings and quality improvement in the food industry.

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Ice-nucleation-active bacteria

Strains of *Pseudomonas syringae* were first observed to catalyze ice formation in supercooled water in 1974

(Ref. 8). Shortly afterwards, strains of *Erwinia herbicola*[9], *Pseudomonas fluorescens*[10], *Erwinia ananas*[11], *Pseudomonas viridiflava*[12], *Xanthomonas campestris* pv. *translucens*[13] and *Erwinia uredovora*[14] were also demonstrated to be ice-nucleation-active (INA) at temperatures in the range –2 to –5°C. All of these bacterial strains are Gram-negative and phytopathogenic (no proof of animal pathogenicity has been reported). They live on plant surfaces, especially on the leaves, where they act as strong heterogeneous ice nuclei in dewdrops, and the ice crystals thus formed grow and break the plant tissues, causing frost injury to the host plants. The ice-nucleation activity of bacterial cells may be influenced by many factors, such as the concentration of the cells, growth medium, growth temperature, physiological state, mineral composition and pH (Refs 14, 15 and J.K. Li and T-C. Lee, unpublished data 1995). Strains of *P. syringae*, *E. herbicola*, *E. ananas*, *X. campestris* and/or their active fractions have been tested in some model food systems and some real foods to examine their effects on the freezing process. A detailed description of their applications will be given in a later section.

Ice-nucleation genes and their products

In every strain investigated so far, bacterial ice nucleation is determined by a single gene that codes the Ina protein. To date, at least six DNA fragments imparting the Ina$^+$ phenotype to *Escherichia coli* have been sequenced and published (Table 1)[16–21]. The genes have been mapped to a small region on the chromosomal DNA of the bacteria, and range in size from 3.4 kb to 7.5 kb. An imperfectly repeated DNA sequence corresponding to the repeated octapeptide AGYGSTLT is found in all of the genes[4]. It was also found that the level of ice-nucleating activity in the transformants correlated with the level of protein expression[22]. The protein sequences inferred from the DNA sequences are more strongly conserved than the DNA sequences themselves.

The translation product of the *inaZ* gene from *P. syringae* was predicted to contain 1200 amino acids and have a molecular weight of ~120 kDa, comprising exactly 122 contiguous repeats of the consensus octapeptide[19]. The ice-nucleation proteins from the various bacteria with the Ina$^+$ phenotype have similar primary structures (Fig. 1)[4]. Each protein consists of three distinguishable domain structures: a unique N-terminal domain (comprising ~15% of the total sequence), which is relatively hydrophobic; a unique C-terminal domain (4%), which is rich in basic residues and is very hydrophilic; and a central repeating domain (81%), which is hydrophilic and particularly rich in alanine, glycine, serine and threonine, and which can be subdivided into three regions. (The first two of these repeat with high fidelity of a period of 48-peptide units; each 48-peptide unit can be further divided into three 16-peptide repeat units of medium fidelity; again, each 16-peptide unit can be further divided into two 8-peptide repeat units of low fidelity, in which only two of the eight peptides are strongly conserved. The third region of the repeating domain contains only an 8-peptide period of repetition.)

The secondary structure of the protein is not known; various structural models have been proposed[23–25], all of which suggest that the ice-nucleation protein serves as a template for orienting water into an ice crystal.

Table 1. *ina* genes that have been sequenced

Gene	Strain	Size (kb)	Ref.
inaA	*Erwinia ananas* IN-10	4.3	16
inaU	*Erwinia uredovora* KUIN-3	3.4	17
inaW	*Pseudomonas fluorescens* MS1650	7.5	18
inaZ	*Pseudomonas syringae* S203	4.4	19
iceE	*Erwinia herbicola* M1	4.9	20
inaX	*Xanthomonas campestris* pv. *translucens* X56S	5.3	21

Components of INA sites

It is generally believed that the bacterial ice-nucleation protein plays a central role in the ice-nucleation activity. However, to date, none of the Ina proteins have been successfully purified from their native producing bacterial strain. The overexpressed protein of the *inaA* gene from *E. ananas* IN-10 was shown to be present in inclusion bodies in *E. coli*, and was purified to apparent homogeneity, by extracting it four times with 2% Triton X-100, in the form of an insoluble precipitate[26]. This protein, with a molecular weight of 130 kDa, was demonstrated to have ice-nucleation activity above –5°C, even in the absence of membrane components such as sugars and lipids. The protein of the *inaZ* gene from *P. syringae* S203 has also been identified and purified following overexpression in *E. coli*[27]. This protein is active only at –13.2°C, and has an apparent molecular weight of 153 kDa, which is 34 kDa higher than the molecular weight of 119 kDa predicted from the *inaZ* gene sequence. Experimental evidence, based on the effects of specific enzymes (e.g. *N*- and *O*-glycanases, α- and β-mannosidases and β-galactosidase) or chemical probes (e.g. lectins, chelating compounds, borate compounds and sulfhydryl reagents), indicated that the most effective ice-nucleation structures may be lipoglycoprotein complexes[28,29]. Phosphatidylinositol was also identified as a major component of the ice-nucleating site on the outer surface of two bacteria, *P. syringae* and *E. herbicola*, based on the effect of the inhibition of plant lectins and phosphatidylinositol-specific hydrolase on ice-nucleation activity. Extracts of these two Ina$^+$ bacteria displayed phosphatidylinositol synthase activity, whereas extracts from related Ina$^-$

Pseudomonas or *Erwinia* strains did not[30].

Erwinia herbicola was the first bacterium to be shown to produce extracellular ice-nucleating matter, which is proteinaceous and associated with membrane vesicles[31]. Extracellular ice-nucleating matter from *E. uredovora* KUIN-3 was purified to apparent homogeneity by ultrafiltration, sucrose density-gradient ultracentrifugation and gel filtration, and comprised 10% lipid, 43% protein, 35% saccharide and 12% polyamine[32]. Further studies suggested that polyamines (e.g. putrescine, cadaverine and spermidine) were the most important component of the ice-nucleating material in this bacterium[33]. De-lipidation of partially purified outer membranes of *P. syringae*, using various de-lipidating agents such as phospholipase A_2, sodium cholate and sodium dodecyl sulfate (SDS), resulted in a significant loss in the ice-nucleation activity associated with the cell envelopes of this and other INA bacteria[34]. Therefore, the authors predicted that a phospholipid component is a requirement for the expression of ice-nucleation activity both in *P. syringae* and *in vitro*.

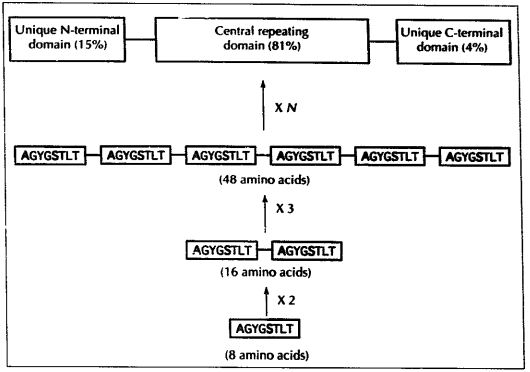

Fig. 1

A schematic representation of the primary structure of a typical bacterial ice-nucleation protein. The protein structure consists of three distinguishable domains: a unique N-terminal domain, a unique C-terminal domain and a central repeating domain. The central repeating domain has three levels of periodicity as depicted below the entire sequence shown at the top of the figure: a primary repetitive sequence consisting of an octapeptide, superimposed by 16-peptide repeats, and again by 48-peptide repeats (that is, the 48-peptide repeats have higher fidelity than the 16-peptide repeats, which have higher fidelity than the 8-peptide repeats). Amino acids are represented by one-letter codes; amino acids **A** and **S** (bold) are more conserved than the other amino acids in the sequences. (Adapted from Ref. 4.)

We have found that the compositional analysis of INA sites was undermined by two factors: first, the sensitivity and accuracy of the assay used to determine ice-nucleation activity; and second, the purity of the INA sites. Furthermore, we believe that the so-called ice nucleus is a poorly defined concept; moreover, its activity measurement and calculation is based on a method derived for inorganic materials[35]. The suitability of this method to the study of biogenic ice nucleation is still subject to verification. In addition, the quantitative relationships between ice-nucleation activity and cell number or material concentration, volume of water drops, holding time of water drops at certain supercooling temperatures, etc. have yet to be established (J.K. Li and T-C. Lee, abstract in 1995 Institute of Food Technologists' Annual Meeting *Book of Abstracts*). Consequently, the exact chemical components of active ice-nucleation sites definitely need to be investigated further.

Purification of the extracellular INA material is presently being carried out in our laboratory; INA material is precipitated from the supernatant of *E. herbicoia* culture broth by adding ammonium sulfate to 50% saturation. The solubilized precipitate is subjected to gel filtration using Sepharose Cl-2B (Pharmacia Biotech Inc., Piscataway, NJ, USA). The INA fraction corresponding to the void volume of the column has an apparent molecular weight of ≥1000 kDa. However, the protein has a molecular weight of only 65 kDa or lower when immunologically detected with an anti-InaA antibody following SDS–polyacrylamide gel electrophoresis (the antibody was kindly provided by Prof. Arai[26]; J.K. Li and T-C. Lee, unpublished data 1995). The further study and characterization of this protein and its mode of action are presently under way.

Freeze-texturing with Ina⁺ bacteria

Arai and Watanabe[36] studied the freeze texturing of food materials using *E. ananas* cells. When the bacterial cells were added to isotropic aqueous dispersions of hydrogels composed of proteins and polysaccharides, the bulk of the water was converted into directional ice crystals at subzero temperatures not lower than –5°C, and resulted in the formation of anisotropically textured products; raw egg white, bovine blood, soybean curd, milk curd, aqueous dispersions or slurries of soybean protein isolate, agar hydrogel, cornstarch paste, and hydrogels of glucomannan and calcium-bridged glucomannan were textured successfully. The flake-like texture of egg white products prepared by the above-mentioned freeze-texturing process was significantly improved as compared with that of products made in a similar manner but textured without the use of Ina⁺ cells. This work demonstrated that the Ina⁺ bacterial cells inhibited supercooling of the bulk of the water in

aqueous dispersions and hydrogels of food proteins and polysaccharides, and also that this changed the ice-crystallization process to improve their textures. Thus, the authors proposed a new process for the freeze texturing of protein products such as soybean curd using *E. ananas* cells as heterogeneous ice nuclei[36].

Efficient freezing with Ina⁺ bacteria and/or their products

Previous work in our laboratory has shown that *P. syringae* cells are efficient ice nucleators when added to foodstuffs, such as fish muscle and other proteins, and sugar and oil solutions (J.M. Ryder, PhD thesis). Whole fish muscle was divided into control groups and groups to which a dispersion of *P. syringae* at a concentration of 10^7 colony-forming units per ml was applied. The treated groups were sprayed with ~0.1 ml of the dispersion per g of fish muscle. Freezing curves for the treated and control samples were determined. As illustrated in Fig. 2, the freezing point (the temperature at which freezing occurs) for salmon muscle is around –1°C (T_1); the nucleation temperatures (the minimum temperature attained by the sample before freezing begins), however, are different for the treated and control samples, that is –1.5°C (T_2) and –4.9°C (T_3), respectively. This means that the degree of supercooling (the temperature difference between the freezing-point temperature and the nucleation temperature of the sample) was reduced by 3.4°C in this case. The total freezing time (defined as the time elapsed from the instant the sample passed through its freezing-point temperature to the instant it reached the freezer temperature) for salmon muscle (e.g. from –1°C at time t_1 to –5°C at time t_3) was reduced by 33% compared with the sample treated with the bacterial cells (time t_2) when frozen at –5°C (Fig. 2). The application of Ina⁺ *P. syringae* cells was also observed to cause the whole fish to be frozen consistently at –5°C within several hours, whereas up to 33% of the untreated fish did not freeze (J.M. Ryder, PhD thesis). In other words, the guaranteed freezing of fish samples within a set time was clearly demonstrated in this case. The degrees of supercooling were also significantly reduced when the bacterial cells were added to other foods, such as sucrose solutions, concentrated fruit juices, surimi samples, egg white, coconut oil, safflower oil, milk and dairy products (J.M. Ryder and T-C. Lee, abstract in 1988 Institute of Food Technologists' Annual Meeting *Book of Abstracts*; M.P. Izquierdo, MSc thesis, University of Rhode Island, Kingston, RI, USA, 1989). It was extremely interesting to note that with some model food systems, such as 35% sucrose solution, ice nucleation and freezing occurred at –6°C with the addition of *P. syringae* cells; however, the control solution without the cells did not freeze under the same conditions. The degrees of supercooling of samples of egg white (1–9% solutions) were reduced by ~4–5°C compared with the untreated samples. The reduction in total freezing time ranged from 12% to 33% as compared with the control samples. Some of the typical effects are presented in Table 2. The reduction in the degree of supercooling for the freezing of soybean curd, cornstarch, egg white, glucomannan, etc. with *E. ananas* cells has also been observed by Arai and Watanabe[36]. The reduction of the degree of supercooling by Ina⁺ bacterial cells and/or their products implies that there is great potential for shortening freezing times, savings in refrigeration costs and efficient production. This means that the energy savings for frozen foods involves at least two aspects: first, the savings made during the freezing process as a result of the total freezing time being shortened; second, the savings made during the storage of frozen foods. For example, a frozen food plant may be able to operate at –30°C rather than at –40°C, because the use of Ina⁺ cells and/or their products will guarantee that the foods freeze at a relatively higher temperature. If this plant operates at 5000 kW, the difference of electrical loads between these two temperatures will be (5000/1.69–5000/1.89 = 2958 kW–2645 kW) 313 kW. If this plant is operated 24 h per day, 260 days per year, and energy costs 10 cents per kW·h, the savings from the cost difference will be ~$195 312 per year.

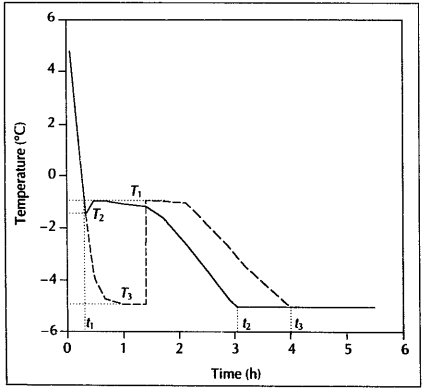

Fig. 2

Typical freezing curves of salmon muscle with (—) and without (– – –) the addition of Ina⁺ *Pseudomonas syringae* cells during storage at –5°C. While the freezing point (T_1) of salmon muscle was approximately –1°C, the nucleation temperature of salmon muscle was raised from –4.9°C (T_3) to –1.5°C (T_2) by the addition of *P. syringae* cells. The total freezing time (as defined in the text) for the salmon muscle with and without *P. syringae* cells was ($t_2 - t_1$) and ($t_3 - t_1$), respectively, and was thus shortened by almost 33% by the addition of *P. syringae* cells (redrawn from J.M. Ryder, PhD thesis; see text for details).

Collette and Lee investigated the freezing effects of a crude membrane fraction from *P. syringae* (prepared by pressurization of a cell suspension and sucrose density-gradient ultracentrifugation) (R.D. Collette and

T-C. Lee, unpublished data 1990), and found that the total freezing time of distilled water containing the crude membrane fraction was reduced by 57%, as compared with the untreated water sample. However, the activity of the crude membrane fraction was much lower than that of intact bacterial cells. The sizes of the ice crystals in treated and untreated ice cream were evaluated by light and electron microscopy. However, the evaluation of ice-size reduction was inconclusive because of the limitations of the methodology used. Zheng and Lee have found that extracellular INA material from *E. herbicola* similarly elevates nucleation temperatures (e.g. reduces the degree of supercooling) and shortens the freezing time when used to assist the freezing of similar model food systems as those mentioned above (Y. Zheng and T-C. Lee, abstract in 1990 Institute of Food Technologists' Annual Meeting *Book of Abstracts*).

Table 2. The effects of Ina⁺ *Pseudomonas syringae* cells and INA fractions from *Erwinia herbicola* on the nucleation temperatures and the total freezing time of various foods[a]

Food samples[b]	Nucleation temperatures (°C) Control	With Ina⁺ cells	With INA fractions	Reduction in total freezing time (%)[c]
Sucrose, 10% (w/w)	DNF	–1.8	–2.6	12
Egg white, 9% (w/w)	–5.1	–0.6	–3.8	19
Safflower oil, 20% (w/w)	–6.0	–0.8	–1.3	16
Salmon muscle	–4.9	–1.5	ND	33

[a] Data from J.M. Ryder, PhD thesis, M.P. Izquierdo, MSc thesis and Y. Zheng, unpublished
[b] Food samples were subjected to freezing at –6°C or in the case of salmon muscle at –5°C for the tests
[c] The reduction in total freezing time was based on the data for samples to which INA fractions had been added, which were extracted from the cell-free supernatant of *E. herbicola* culture broth, except the salmon muscle, which was based on the addition of cells
DNF, Did not freeze
ND, Not determined

Freeze drying and freeze concentration

Erwinia ananas cells were applied to the freeze drying of food items such as soy sauce and soybean paste, which are difficult to freeze under normal conditions; such high salt-containing food items are usually diluted for effective freezing[37]. The addition of Ina⁺ cells made it possible to shorten the freezing times of these foods and to obtain powdered freeze-dried products with greater efficiency. Contact of the cells with the container materials was found to influence the nucleation temperatures; thus, cells of *E. ananas* were entrapped in calcium alginate to prepare an INA gel for freeze concentration[37]. Both the position of ice nucleation and ice growth in the product were arbitrary when the INA gel was used. Before cooling a liquid material such as egg white, the INA gel was placed at a desired position in the liquid, and ice began forming at exactly this position. Following freezing, the ice containing the INA gel was separated easily from the unfrozen material with a sieve until a desired solid content was obtained. By this method, the concentrated raw egg white was found to form a hard gel when heated and to give a fine foam when whipped, and was therefore superior to conventionally produced products. Another preliminary treatment of Ina⁺ bacteria was the application of high-pressure sterilization to the cells before they were used in the freeze-concentration process. Examples of cells that can be used for freeze concentration are *E. ananas* IN-10 and *X. campestris* INXC-1 cells[7]. The use of Ina⁺ *X. campestris* may be preferred from a hygiene point of view because it is a non-phytopathogenic strain. Fresh milk, lemon juice and strawberry paste have also been successfully concentrated using this technique, and were found to have retained their flavor properties better than vacuum-concentrated control samples[7,38]. This aspect of the work has been reviewed elsewhere[7].

Future work

Previous investigations have shown that the use of Ina⁺ bacterial cells and/or their active fractions in food products can elevate the initial freezing temperature; this can result in less energy being utilized and an improvement in the quality of the frozen food products, possibly owing to a shortening of the freezing time, an increase in the freezing rate and the production of smaller, less-damaging ice crystals[2]. We believe that the use of these bacterial ice nucleators is a unique application of biotechnology, as it directly improves the freezing process (Table 3). Generally, further research is needed to improve our understanding of the basic mechanisms, the practical applications and the safety of using this type of material in the commercial freezing of food products.

At present, the further understanding of bacterial ice nucleation relies largely on the ability to produce pure INA materials and the quantification of their activity, which are still far from satisfactory. It is apparent that additional research is still needed to define the bacterial 'ice nucleus' in terms of the relationship between

Table 3. Application of Ina⁺ bacteria and/or their products in food processing

Application	Example foods	Refs
Freeze texturing	Egg, gel, milk, soybean curd	36
Freezing	Fish, surimi, egg, sugar, oil, juice, starch, agar gel, ice cream	See text
Freeze drying	Soy sauce, soybean paste	7, 37
Freeze concentration	Milk, egg, juice	7, 38

molecular structure and function. The quantitative relationships between the activity and the variable parameters that affect the measurement of activity should be established before the mechanism of bacterial ice nucleation can be distinctly elucidated.

Bacterial ice nucleators must be robust and environmentally safe, as well as nontoxic, non-pathogenic and palatable if they are to be used in the food industry. To satisfy these requirements, the ice nucleators must be purified to a biochemically simpler form, such as cell-free preparations and/or extracellular INA preparations, which may also be accepted more readily by food safety regulatory agencies. This involves the enhancement of high levels of ice-nucleation protein in the bacteria; then purification and concentration to an active form. It is hoped that this will alleviate the safety concerns surrounding the application of bacterial ice nucleators in foods. The isolation of edible food-grade Ina$^+$ microorganisms, such as *X. campestris*[7], and the enhancement of their activity may be an alternate means of solving this problem. In 1994, high-pressure-sterilized cells of Ina$^+$ *X. campestris* INXC-1 were permitted for food use by the Japanese Ministry of Health and Welfare. One Japanese company, QP Co. in Tokyo, has also produced pressurized dead cells of *X. campestris* INXC-1 for food processing (M. Watanabe, pers. commun.). Another possible solution is to clone the ice-nucleation gene into edible food-grade microorganisms, such as strains of *Lactobacillus* and/or yeast, which could be used directly in foods.

Sensory evaluation is recognized as one of the most reliable methods for estimating food quality; however, this type of evaluation has not yet been performed on products processed with the application of Ina$^+$ cells and/or their cell-free preparations because of the above-mentioned safety concerns. It is obvious that additional work to demonstrate the energy savings and product quality improvements that may be achieved by the use of the cell-free bacterial ice nucleators or functionally equivalent preparations and/or edible food-grade Ina$^+$ phenotype microorganisms in various model food systems and real foods both at the laboratory bench-top scale and pilot scale, is essential before the final commercialization of their profound application in the food industry.

On comparing the freezing processes currently used in the food industry, there is an obvious trend towards quicker freezing techniques such as those involving fluidized beds and cryogenics to preserve high-quality frozen foods[2]. The relatively high cost of such operations has been the major obstacle to their widespread use. At present, the trade-off between the rate of freezing and the cost of freezing may be balanced by using a combination of freezing processes such as cryogenic and mechanical techniques. The application of bacterial ice nucleators to food products in the pre-freezing regime represents an additional approach that could be used to significantly increase the cost-effectiveness of these processes, enhance the quality attributes of frozen foods and provide additional impetus for further growth of this market sector. In addition, it may be more straightforward to use bacterial ice nucleators for pre-freezing treatment in the freeze-drying and freeze-concentration processes to bring about energy savings and product quality improvements. A critical benefit–cost analysis of these processes should also be investigated.

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Review

Visions in the mist: The *Zeitgeist* of food protein imaging by electron microscopy

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The biological activity of a protein is a function of its three-dimensional structure, as well as its interaction with other molecules. This applies equally to foods containing proteins whose functional properties (e.g. solubility, gelation, foaming and emulsification) are dictated primarily by their structures. High-resolution transmission electron microscopy (TEM) is an appropriate tool for determining the structures of biological macromolecules and their complexes, especially when they cannot be studied by X-ray crystallography. Although TEM and appropriate image analysis techniques have been used primarily in molecular structural biology, we have recently adopted this combination into the realm of food science. The principles of TEM, including sample preparation and image analysis techniques, as well as the potential benefits that high-resolution TEM may bring to food protein chemists working in the area of protein structure–function relationships will be discussed in this article.

Germane to the optimal use of both existing and future proteins in food commodities is a better understanding of the nature of the relationship(s) between structure and function. Critical to this understanding is the accurate determination of the three-dimensional (3D) structure of a protein; however, this is one of the most difficult tasks facing protein chemists. X-ray crystallography is unequivocally the most accurate means for structure determination, allowing atomic resolution (>0.3 nm) of protein structures. However, many proteins, particularly food-related ones, do not form crystals of suitable size and order for X-ray diffractometry; thus, TEM represents an appropriate alternative approach for determining protein structure. Recent and continuing advances in TEM-related technology (primarily sample preparation) and image analysis now render this approach capable of determining protein structure to near atomic resolution[1–3]. This article reviews some of the new developments associated with TEM, and their potential application to food science.

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Electron imaging of proteins

Modern transmission electron microscopes have an instrumental resolution usually approaching and often exceeding 0.3 nm. Yet, the determination of protein structures to such resolution cannot usually be obtained using TEM. The limitations lie not with the instrumentation, but rather with the sample. Proteins exist in a solvated form in their native state, often with water as an integral part of their structure. The surface tension forces of the receding water droplet often disrupt the structure of the sample as it is dried in preparation for TEM. Proteins consist primarily of light elements: carbon, nitrogen, oxygen and hydrogen. The high energies of the electron beam (usually 80 keV) disrupt the specimen while it is being imaged. Moreover, the contrast of a protein molecule lying on a carbon support is very poor. Heavy atom salts are then required to stain the protein selectively to enhance its visualization, but this limits the resolution[4]. Finally, electron images of a