Biomedical Data Mining

Extended Abstract

Application of new quality products of type I–III antifreeze proteins and antifreeze glycoprotein

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Abstract

A regular ice block is made out of various single ice precious stones that are made in water during freezing. The precious stones develop and consolidate to frame an ice block, if the temperature stays underneath 0°C. Type I-III radiator fluid proteins (AFPs) and liquid catalyst glycoprotein (AFGP) collect on the surfaces of early stage ice gems to hinder their development and combining, bringing about a total of minuscule ice precious stones rather than an ice block. This capacity of AFP will be valuable for the conservation of an assortment of water-containing materials like prepared food sources, soups, frozen yogurts, noodles, breads, vegetables, seeds, drinks, liquor, medications, makeup, gels, cells, tissues, and organs. In keeping the size of each ice precious stone to a base, AFP use may significantly improve the adequacy of protection. Likewise, fish-determined AFPs tie to the lipid bilayer to drag out the lifetime of cells under hypothermic condition (+4°C), a capacity that might be appropriate to momentary cell conservation or "cell stopping". It ought to be noticed that every AFP and AFGP test is consistently a combination of 2-13 isoforms, what work together undeniably more adequately than any single isoform. We have hence evolved arrangement strategy for quaity results of fish type I-III AFPs and AFGP, and inspected their materialness in both modern and clinical fields. An illustration of use is manufacture of exceptionally permeable material utilizing "gelation and freezing strategy". In this technique, we initially set up an answer containing gelatin, fired powder, and AFP to be cooled to frame a cylindical state of gel. This AFP-containing gel is then positioned on a frozen plate to incite unidirectional freezing. Since AFP ties onto the side (crystal plane) of the stretching ice gems, incredibly honed and consistently adjusted ice needles are made in the frozen gel. Subsequent to sintering at 1,000°C, a clay containing various unidirectionally-adjusted dendritic pores is made. The quality results of AFP and AFGP may understand further developed methods that have been normal by numerous incredible pioneers of this field.

Introduction

Concentration dependence is a significant factor for all protein studies and their applications. Protein concentration varies from almost completely insoluble to hundreds of milligrams per milliliter. An example of an insoluble protein is crambin, whereas serum albumin is one of the highly dissolved proteins (>500 mg/mL). Both intrinsic and extrinsic factors are known to affect solubility. Examples of intrinsic factors are the primary to tertiary structures of proteins with a hydration shell, although their relationship with solubility is not perfectly understood. The extrinsic factors include ionic strength, pH, temperature, and buffer detergents. In this study, we evaluated the solubility limit of native antifreeze proteins (AFPs) and antifreeze glycoprotein (AFGP) using 4 °C water, and examined how their ice-binding activity responds to the change in concentration. Distilled water was used as the solvent in all experiments. The X-ray structural coordinates including the hydration waters are available for AFPs.

AFPs and AFGP have been purified from various cold-adapted fishes living in northern midlatitude waters. These include AFP I from barfin plaice (Liposetta pinnifasciata), AFP II from longsnout poacher (Brachyopsis rostratus), AFP III from notched-fin

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eelpout (Zoarces elongatus Kner), and AFGP from saffron cod (Eleginus gracilis). A technique to obtain massive amounts of these proteins has been developed, which utilizes the muscle homogenate of each fish as a source material. For AFP I–III, the obtained samples were found to consist of a mixture of the isoforms, in which the primary sequence of the dominant species was determined. Three-dimensional (3D) structures were also determined for each AFP isoform. For example, AFP I is an alanine-rich amphipathic α-helical polypeptide (Mw = 3.5 kDa), AFP II is a disulfide-bond-rich globular protein exhibiting high structural similarity with a carbohydrate-recognition domain of C-type lectin (Mw = 14 kDa), and AFP III is another globular protein composed of twisted loops folded into triple-strand β-sheets (Mw = 6.5 kDa). The AFGP consists of tripeptide repeats (Ala–Ala–Thr)n (n = 4–50), in which Cβ of Thr is glycosylated with a disaccharide β-D-galactosyl-(1→3)-α-N-acetyl-D-galactosamine. Hence the molecular weight of AFGP ranges from 2.6 kDa (n = 4) to 33.7 kDa (n = 50). The AFGP purified from saffron cod consists of at least 18 distinct isoforms, in which a few amino acids are replaced with Arg or Pro. Although X-ray structure was not determined for AFGP, a structural motif called "polyproline type II helix" was hypothesized on the basis of its circular dichroism (CD) spectra and solution nuclear magnetic resonance (NMR) data.

When approximately 10 mg/mL solution of AFP or AFGP is placed in a freezer for example, neither of them turns into a normal ice block, but instead transforms into an assembly of numerous dispersive single ice crystals. The growth of each crystal is inhibited, as the antifreezes adsorb onto each crystal surface via an "adsorption–inhibition mechanism". The efficiency of ice growth inhibition is evaluated using thermal hysteresis (TH), the difference between the melting and freezing points of a solution (Tm and Tf, respectively), which is controlled according to the types and concentrations of AF(G)Ps. In previous studies, 0.6–1.0 °C of TH was determined for AFP I–III at the concentration of approximately 10 mg/mL, although their solubility limit was not closely examined. The TH values of only 0.01–0.03 °C were evaluated for AFP mimetics, such as 50 mg/mL solution of polyvinyl alcohol (PVA). A relatively high TH value of 1.4 °C was evaluated for an approximately 7.5 mM (25 mg/mL) solution of AFP I. A lower solubility was suggested for AFP III, so that a hydrophilic tetrapeptide sequence KDEL was appended to the C terminus of the AFP III isoform for NMR studies. This mutant's TH value is almost the same as that of wild-type (~0.8 °C at 6 mg/mL). In contrast, approximately 1.2 °C of maximal TH value was evaluated for a 40 mg/mL solution of AFGP, suggesting its superior solubility,

A recent study showed that a native AFP I sample purified from barfin plaice is extremely soluble (~650 mg/mL), and exhibited high TH activity (3.0 °C) at 200 mg/mL. The study showed that this AFP I binds to only a limited area of an ice crystal at lower concentrations (<0.01 mg/mL); however, it expands the target area to the whole crystal surface when the concentration is increased up to 0.1 mg/mL. This prompted us to question whether high water solubility is a common property of fish AFPs and AFGP, and how their ice-binding activity changes in response to the increase in concentration. Computational approaches and structural biology pointed out that an ice-binding site (IBS) of AFP accompanies a unique hydration shell including some waters organized into an ice-like arrangement. Such IBS formation was suggested for AFPs from bacteria, fungi, and fishes, but not those from insects. The ice-like waters were hypothesized to merge with, and freeze to, an intrinsically disordered water layer constructing the surface of ice, leading to AFP–ice complex formation. Davies and colleagues named this mode of AFP function the anchored clathrate water mechanism. The present study examined the water solubility, TH value, and fluorescence-based ice plane affinity (FIPA) of each sample, and results are

considered in relation to the anchored clathrate water mechanism.

Materials and Methods

Preparation of Antifreeze Proteins and Antifreeze Glycoprotein

The fish species barfin plaice (Liposetta pinnifasciata), longsnout poacher (Brachyopsis rostratus), notched-fin eelpout (Zoarces elongatus Kner), and saffron cod (Eleginus gracilis) were collected by Nichirei Corporation. Size exclusion chromatography and anion exchange chromatography were successively performed for the prepared crude sample powders with a Sephadex G-25 size-exclusion column (XK 50/30, 500 mL; GE Healthcare Life Sciences, Pittsburgh, PA, USA) and DEAE Sepharose anion-exchange column (XK 50/20, 80 mL; GE Healthcare), respectively. The purified samples were dialyzed against Milli-Q water for three overnights, and then lyophilized for frozen storage. The purity of the final product was checked with 15% SDS–PAGE using a minislab electrophoresis kit (AE-6500; ATTO Corp., Tokyo, Japan).

Evaluation of Protein Concentration

The lyophilized powder of each AFP I–III and AFGP was weighed and dissolved with 200 µL of distilled water in a 50 mL centrifuge tube. The respective samples were then placed into NMR tubes (Ø = 5 mm), where they were left overnight at 4 °C, and photographs of the samples were taken. Approximately 400 mg/mL (AFP I), 200 mg/mL (AFP II), 100 mg/mL (AFP III), and >1800 mg/mL (AFGP) of the concentration limit were evaluated at 4 °C; results showed that no significant precipitants were produced below these concentrations. The weight-base evaluation was further verified using a fluorescence method (Qubit Protein Assay Kit; Thermo Fisher Scientific, Waltham, MA, USA). We prepared 2–15 µg/mL protein samples to be reacted with a dye reagent provided in this kit. The correlation between protein concentration and the fluorescence emission was calibrated by standard protein solution supplied by the kit. We confirmed that the estimated protein concentration by the kit was consistent with weight-based concentration by applying another protein standard (chicken lysozyme).

Analysis of Fluorescence-Based Ice Plane Affinity

For the FIPA experiment, several single ice crystals with a cylindrical shape (Ø = 2–3 cm) were initially prepared within the plastic pipes (l = 4 cm) according to a previously described procedure [27,28,29]. After determination of their c-axis on a polarizer, each cylindrical ice was half-cut and mounted on a hollow copper tube (Ø = 15 mm), in which −0.8 °C coolant was circulated by a refrigerant pump (Hitachi AMS-007; Hitachi, Tokyo, Japan). The cylindrical ice crystal was then immersed in distilled water for overgrowth to be changed into a hemispherical shape. We prepared many such spherical single ice crystals, and immersed each crystal in a solution of fluorescent AFP II or AFGP, whose concentration was adjusted between 0.01 and 0.1 mg/mL. After 2 h of incubation of each crystal in a solution, the fluorescence emission or the FIPA pattern was observed under UV light. After capturing the snapshots, a six-sided star mark was created in the center of each spherical ice with the ice-pitting protocol , which determined the a1–3-axes of each single ice crystal.

Ethical Approval

All methods were carried out in accordance with relevant guidelines and regulations of National Institute of Advanced Industrial Science and Technology (AIST), Japan. All experiments involving animals were conducted with approved methods designated in Guidelines for Proper Conduct of Animal Experiments, Science Council of Japan (Low No. 105, 1973).

Results and Discussion

Sample powders of AFP I–III and AFGP of 20–30% purity were obtained from Nichirei Corporation (Tokyo, Japan), and were extracted from the muscle homogenates of barfin plaice, longsnout poacher, notched-fin eelpout, and saffron cod, respectively. Size exclusion chromatography followed by anion exchange chromatography were performed on 6 g of each crude powder according to previously described procedures, which yielded approximately 1500, 200, 130, and 1600 mg of lyophilized powders of AFP I–III and AFGP, respectively. Then, chromatography was performed

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again to obtain the gram order of the samples to be analyzed with high-performance liquid chromatography, which gave us several isoform fractions. The bipyramidal ice crystals were observed for each sample as a typical sign of their ice-binding ability. Their electrophoretograms with 15% tricine sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) exhibited a typical major band (supporting information S1) attributable to their isoform mixture. The analysis of SDS–PAGE bands using Image-J (https://imagej.nih.gov/ij/) enabled us to know that our samples are purified to 93–97% homogeneity.

The consistency between the weight-base and the fluorometer-base concentrations was verified for AFP I–III within an error range of 1–12% (Supporting Information S2). An ultimately concentrated solution of AFGP (>1800 mg/mL) generated no precipitant but exhibited significant viscosity similar to that of high molecular weight polymers; they may become viscous by means of a strong internal friction between the randomly coiled swollen polymers with surrounding solvents. For proteins, precipitants and/or viscosity of the condensed solution have been assumed to originate from more complex factors, such as hydration, surface charge, hydrophobicity, and 3D structure, as they alter the manner of protein–protein interaction. It is significant that when the AFP I solution was incubated at 25 °C for 12 h, it became highly viscous and changed into a gel state. This AFP I gel was changed into a solution state when the temperature was decreased to 4 °C. The viscosity of the AFGP solution became more significant, and the solubility of AFP II and III was lowered to 20–30 mg/mL when they were incubated at room temperature. These results suggest that native AFP I–III and AFGP samples are highly soluble only when they are dissolved in chilled water. An AFP molecule generally forms a hydrophobic surface that locates several polar residues to construct the ice-binding site. The temperature increase theoretically strengthens the hydrophobic interaction between AFPs in solution, which might be a reason of the precipitation and lower solubility of AFPs and AFGP at higher temperature.

Thermal Hysteresis Measurement for the Native Samples

TH is defined as the difference between the Tm and Tf of an AFP solution. The |Tf| value is also called freezing-point depression. Differential scanning calorimetry (DSC) and sonocrystallization method were the methods used to determine |Tf| via detection of the latent heat emission that originates from the number of crystals generated at the moment of freezing. In contrast, a method that uses a photomicroscope system equipped with a cooling stage to determine the growth-initiation temperature and melting temperature of a single ice crystal was also introduced. The two respective temperatures are equal to the Tf and Tm of an AFP solution. The TH value (i.e., TH = Tm − Tf) is the temperature range in which an AFP-adsorbed single ice crystal neither grows nor melts. The TH was therefore used as a parameter to evaluate the AFP's ice-binding ability. It should be noted that the cooling rate (°C/min) and ice crystal size (μm) need to be adjusted before one can compare the TH value between samples. Such a photomicroscopic method was used in this study, for which a key device is shown in Figure 2A. This is a temperature control box (type 10002L; Linkam Scientific, London, UK) covered with a glass plate to be placed on a photomicroscope stage. The temperature of the glass slips holder (Figure 2A) was controlled to be between −198 and 600 °C with an accuracy of ±0.2 °C by mixing the use of liquid nitrogen and an electric heater. A 0.8 μL AFP sample was soaked into a capillary tube (Ø = 0.92 mm) to be set into a homemade copper holder. This was then set into the portion of the glass slips holder to observe the process of sample freezing and/or melting under the beam of the light, thus allowing TH evaluation.

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