Mass preparation of AFPI, AFPIII, and AFGP from Japanese fish

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Abstract

Freezing is initiated by generation of numerous embryo ice crystals in water, which grow and merge together to form their multicrystaline state, the ordinary ice block. Antifreeze protein (AFP) is capable of binding to these ice crystals to inhibit their growth, and disturbs such general ice formation. This mechanism is expected to solve many technical problems with regard to the frozen storage of water-containing materials, such as foods and tissues. The fish-derived AFP can also bind to the lipid bilayer to prolong the lifetime of a cell, which is applicable to the short-term hypothermic cell preservation. Natural fish AFP is a mixture of 2–13 isoforms that function together far more effectively than any single isoform. We have been therefore trying to develop mass preparation method of natural fish AFP (the mixture), and now AFPI, AFPIII, and AFGP samples are available from NICHIREI CORPORATION, Japan (E-mail to s.tsuda@aist.go.jp or directly to N1000X016@nichirei.co.jp). Each AFP sample is highly purified (>95%) and contains neither cations nor buffer detergents. The samples are also sterilized using 0.22 μm syringe filter, so that directly applicable to any kind of experiment including medical tests. AFPII sample will also be released soon.

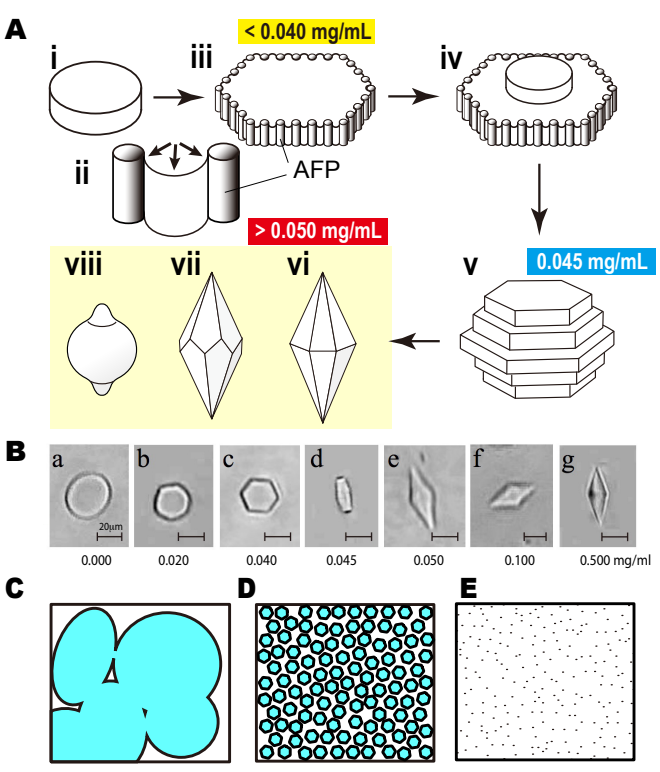
Products

We have been improved the methods to purify the AFP samples from fish muscle homogenates (Nishimiya, 2008). Our AFPI, AFPIII, and AFGP show 800, 50, and 1,600 mg/mL of high water solubility, and ther maximum TH values are 3.2, 2.1, and 5.0°C, respectively. They are also recovered from heating to 95°C. In addition to these highly purified AFPs, crude products of approximately 20% purity can also be supplied on request.

	AFPI	AFPIII	AFGP
	Pleuronectidae	*Zoarcidae*	*Gadidae*
	Ala-rich, 11-res. repeats	Unbiassed, SP and QAE-groups	
	3.3 kDa	7 kDa	2.6 - 34 kDa
	α-Helix	β-Sandwich	Polyproline II helix

Functions

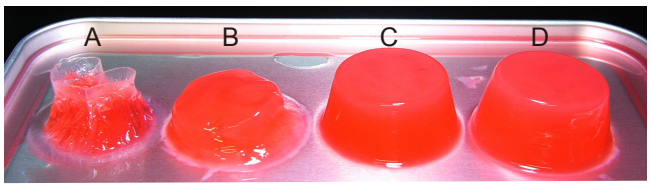
A single ice crystal consists of water molecules in hexagonal arrangement, while in solution it forms a disk-like shape (**A-i**). In AFP solutions, ice crystal growth is only allowed between the bound AFPs (**A-ii**) according to the Gibbs-Thomson effect. The resultant convex ice front sandwiched between the AFPs is terminated its growth to form a flat, AFP-accumulated surface (**A-iii**). This process transfers the disk-shaped ice crystal into a hexagonal plate, and allows generation of a new disk on that plate through a mechanism called 2D-nucleation. Repeated AFP binding and a new disk generation causes successive stacking of smaller hexagonal ice plates in the direction of the c-axis (**A-iV&V**), forming a hexagonal bipyramid (**A-Vi**) onto which millions of AFPs are adsorbed. This unique ice crystal is further modified into its derivative forms, such as hexagonal trapezohedron (**A-Vii**) and a lemon-like shape (**A-Viii**). The ice hexagonal plate remains unchanged and is not further modified into a bipyramid, when AFP concentration is below 40–50 μg/mL (depends on the condition), which we termed the “critical ice-shaping concentration” (**CISC**) (**Mahatabuddin et al. 2016**). The general disk-shape ice crystals (**A-i**) undergo ice recrystallization and forms multicrystalline state by freezing (**C**). This ice expands the volume and physically destroys inside texture of all of the frozen materials. AFP is capable of inhibiting growth of the ice crystals to form ice slurry (**D**). If we can minimize the size of each ice crystal ultimately small, the frozen state shoud become noncrystalline glass-like state (**E**). That is, AFP should be able to freeze and preserve the water-containing materials by filling their inside with numerous tiny ice crystals. This can be realized with a home freezer (-20 degC) without LN_2 nor deep freezer.



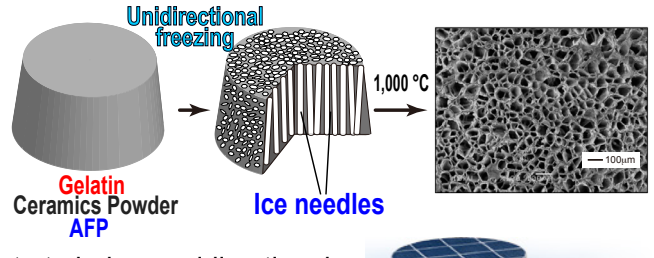
Application 1

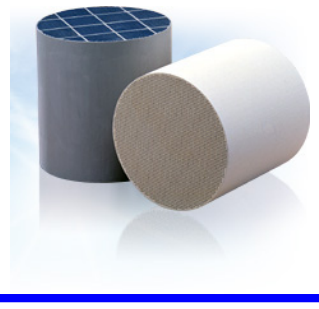
AFP exhibited a gel protection function, which made us to develop “gelation freezing method” to fabricate porous materials, for which AFP has a significant contribution (Fukushima et al. 2013). A solution of gelatin, ceramic powder, and AFP is initially cooled to form a gel and placed on a freezing plate to induce unidirectional freezing. Because AFP binds to the surface of the elongating ice crystals, extremely sharpened and uniformly aligned ice needles are created in the frozen gel. After sintering at 1,000°C, a ceramic containing numerous unidirectionally-aligned dendritic pores is created.

0.5% (w/v) Agarose gel after freeze-thawing



AFP = 0 mg/ml	0.01 mg/ml (0.001w/v %)	0.02 mg/ml (0.002 w/v %)	0.05 mg/ml (0.005w/v %)





Application 2

Cell protection effect of AFP has been suggested in both cryo- and hypothermic conditions. The former used a very diluted solution (below CISC) so as not to create ice bipyramids. In nature, AFP functions with glucose, glycerol, lipids, ions, minerals, etc. The AFP performance will be maximized by optimal combinations of these substances, which should be different between the applications.

Cells	Freezing (Cryo) preservation			References
Rat kidney	*Tm*AFP	-4 ˚C	61.5 μM	Tomalty 2017
Mouse ovary	AFPIII, FfIBP	vitrification	10 mg/ml	Kim 2017
Bovine oocyte	AFGP8	vitrification	1 mM	Liang 2016
Buffaro sperm	AFGP	-196 ˚C	1,000 μg/ml	Qadee 2016
Mouse ovary	AFPIII	vitrification	20 mg/ml	Lee 2015
Marine diatom	LeIBP	-196 ˚C	100 μg/ml	Koh 2015
Buffaro sperm	AFPIII	-196 ˚C	100 μg/ml	Qadee 2014
Rabbit sperm	AFPIII	-196 ˚C	1 μg/ml	Nishijima 2014
Rabbit embryo	AFPIII	-196 ˚C	500 ng /ml	Nishijima 2014
Seabream sperm	AFPI, III	-196 ˚C	1 μg/ml	Beirao 2012
Mouse oocyte	AFPIII	vitrification	500 ng /ml	Jo 2012
Red blood cell	LeIBP	-196 ˚C	800 μg/ml	Lee 2012
Mouse oocyte	AFPIII	vitrification	500 ng/ml	Jo 2011
Seabream embryo	AFPI	- 10 ˚C	N/A	Robles 2007
Human blood cell	syAFGP	-196 ˚C	500 μg/ml	Matsumoto 2006
Rat islet	syAFGP	-196 ˚C	500 μg/ml	Matsumoto 2006
Bovine sperm	AFPI	-196 ˚C	1 μg/ml	Prathalingam 2006
Red blood cell	AFPI, II, III	-196 ˚C	340 μg/ml	Chao 1996
Rat liver	AFGP	-3 ˚C	1 mg/ml	Rubinsky 1994
Red blood cell	AFPI	-196 ˚C	150 μg/ml	Carpenter 1992
Mouse embryo	AFGP	vitrification	40 mg/ml	Rubinsky 1992

Cells	Non-freezing preservation				References
Bovine embryo	AFPI, III	+4 ˚C	10 days	10 mg/ml	Ideta 2015
Rat Insulinoma cell	AFPI, III	+4 ˚C	5 days	10 mg/ml	Kamijima 2013
Mouse spermatozoa	AFPIII	+4 ˚C	5 days	1 mg/ml	Kiga 2011
Rat neurons	AFPI	+4 ˚C	8 hrs	10 mg/ml	Rubinsky 2010
Human hepato cell	AFPIII	+4 ˚C	72 hrs	10 mg/ml	Hirano 2008
Rat heart	AFPI, III	-1.3 ˚C	32 hrs	15 mg/ml	Amir 2004
Carp Spermatozoa	AFGP	+4 ˚C	5 days	10 mg/ml	Karanova 2002
Sheep embryo	AFPI, III	+4 ˚C	4 days	10 mg/ml	Baguisi 1997
Human platelet	AFGP	+4 ˚C	21days	1 mg/ml	Tablin 1996
Human oocyte	AFPI, III	+4 ˚C	48 hrs	1 mg/ml	Rubinsky 1993
Rat liver	AFPIII	+4 ˚C	24 hrs	15 mg/ml	Lee 1992
bovine oocyte	AFPI, II, III	+4 ˚C	24 hrs	20 mg/ml	Rubinsky 1991
pig oocyte	AFGP	+4 ˚C	24 hrs	40 mg/ml	Rubinsky 1990

Summary

Highly purified natural fish AFPI, III, and AFGP containing neither salts nor buffer detergents are now supplied from NICHIREI CORPORATION, Japan. Ref. Nishimiya et al. Synthesiology 2008, **1** (1) 4-17; Mahatabudding et al. Cryobiology and Cryotechnology 2016, **62** (2) 95-103; Fukushima et al. 2013, J Am Ceram Soc **96**, 1029-1031. This research was supported by JSPS (15K13760).

* AFP1 is “BpAFP” introduced in Mahatabuddin et al. 2017 Concentration-dependent oligomerization of an alpha-helical antifreeze polypeptide makes it hyperactive, Scientific Reports 7, 42501.

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