# The Production and Bioactivity Analysis of Type I Antifreeze Protein


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**ABSTRACT**

ABSTRACT Antifreeze proteins (AFPs) were first found in the blood and tissues of fish in the polar region and were characterized by lowering freezing temperature and preventing ice crystal formation, so which help fish to survive under low temperature in the polar region. The aims of this research were to investigate (1) the production capacity and bioactivity of type I antifreeze protein. Type I antifreeze protein sequence of winter flounder was constructed on the pET-28b(?) vector, after transformed into E. Coli, followed by induction by IPTG and purification, its production capacity and effect on the cryopreservation of zebrafish embryos were analyzed; and (2) the cryotolerance of zebrafish with type I AFP on pEGFP-N1 and pAAV-CMV-1RES- hrGFP-Neo vectors. The production of AFP was higher at 2 hours after induction with 0.4 mM IPTG than that at 1 hour ( 33.1 mg vs. 7.4 mg ). In general, the amount of AFP production was increased with culture peroids, however, the production at 3～24 hours showed no significant differences ( 39.9～65 mg, p＞0.05 ). The average concentration of AFP was 65 mg when AFP was induced in one liter of bacterial suspension for 6 hours. Six groups of various concentrations ( 0, 1, 5, 10 and 15 mg/mL ) of AFP and 5% glycerol were applied as cryoprotectants to freeze the zebrafish embryos at -10?C. The survival rate was determined by using fluorescent dye. The results showed the survival rates of 0, 1, 5, 10, 15 mg groups and 5% glycerol group were 70.2%, 82.4%, 81.5%, 83.4%, 84.3% and 90.2%, respectively. Though higer surviral rate was observed in 5% glycerol group, no significant differences existed between groups ( p＞0.05 ). The success of cryopreservation of embryos depends on the optimal concention of AFP and cooling rate. The survival rates of zebrafish embryos injected with different constructions of pEGFP-N1 (control), pEGFP-N1-AFP, pAAV-CMV-IRES-hrGFP-Neo (control) and pAAV-CMV-IRES-hrGFP- Neo-AFP were 58.1%, 60.6%, 43.8% and 34.3%, respectively; and the fluorescence expression rates of pEGFP-N1 and pEGFP-N1-AFP groups were significant higher than those of pAAV-CMV-IRES- hrGFP-Neo and pAAV-CMV-IRES-hrGFP-Neo-AFP groups ( 40.6% and 52.9% vs. 9.6% and 6.1%, p＜0.01 ). Two hundred and seventy six bp were cloned from transgenic zebrafish, from which 120 bp were identified, and had a similarity of 43.4% when compared to AFP sequence. Hopefully, a AFP-transgene zebrafish model can be established through this study, and the technique can be applied to other fishes for preventing them from chilling injury in the fishery in Taiwan.



Keywords : antifreeze protein,microinjection, cryopreservation, zebrafish


**Table of Contents**

## REFERENCES

參考文獻 陳立人、黃文瑛、駱亞欣、吳明哲。1995。第三型抗凍蛋白對極 體形成前後豬卵之冷凍保存效果。畜產研究 28（3）:169-179 。 李廣武、鄭從義、唐兵。1998。第一版。低溫生物學。湖南科學 技術出版社。pp 1～71。 吳明哲、李秀美。1996。豬卵母細胞的玻 璃化冷凍。 中國畜牧學會會誌。第25卷。pp 35～51。 Amir, G., Rubinsky, B., Kassif, Y., Horowitz, L.,Smolinsky, A. K. and Lavee, J. 2003. Preservation of myocyte structure and mitochondrial integrity in subzero cryopreservation of mammalian hearts for transplantation using antifreeze proteins — an electron microscopy study. Eur. J. Cardiothorac. Surg. 24 : 292-297. Arav, A., Rubinsky, B., Seren, E., Roche, J. F. and Boland, M. P. 1994. The role of thermal hysteresis proteins during cryopreservation of oocytes and embryos. Theriogenology 41 (1) : 107-112. Baardsenes, J. and Davies P. L. 2002. Contribution of hydrophobic residues to ice binding by fish type III antifreeze protein. Biochim. Biophys. Acta 1601 : 49-54. Bilton, R. J. and Moore, N. W. 1976. In vitro culture, storage and transfer of goat embryos. Aust. J. Biol. Sci. 29 : 125-129. Bowen, R. A., Reed, M., Schnieke, A., Seidel, G. E., Stacey A.,Thomas, W. K. and Kaujkawa, O. 1994. Transgenic cattle resulting from biopsied embryos: Expression of c-ski in a transgenic calf . Biol. Reprod. 50 : 664-668. Brinster, R. L., Chen, H. Y., Trumbauer, M. E, Yagle, M. K. and Palmiter R. D. 1985. Factors affecting the efficiency of introducing foreign DNA into mice by microinjection eggs. Proc. Natl. Acad. Sci. 82 : 4438-4442. Chan, S. L., Miao, M. Fletcher, G. L. and Hew, C. L. 1997. The role of CCAAT/enhancer-binding protein α and a protein that binds to the activator-protein-1 site in the regulation of liver-specific expression of the winter flounder antifreeze protein gene. Eur. J. Biochem. 247 : 44-51. Chen, T. T. and Powers. D. A.. 1990. Transgenic fish. Trend Biotechnol. 8 : 209-215. Davies, P. L. and Hew, C. L. 1990. Biochemistry of Fish antifreeze proteins. FASEB J. 4 : 2460-2468. Deng, G., Andrews, D. W. and Laursen, R. A. 1997. Amino acid seqence of a new type of antifreeze protein from the longhorn sculpin Myoxocephalus octodecimspinosis. FEBS Lett. 402 : 17-20. DeVies, A. L. 1983. Antifreeze peptides and glycopeptides in cold water fishes. Ann. Rev. Physiol. 45 : 245-260. Drickamer, K. and Taylor, M. E. 1993. Biology of animal lectins. Ann. Rev. Cell Biol. 9 : 237-264. Du, S. J., Gong, Z., Hew, C. L., Tan, C. H. and Fletcher, G. L. 1992. Development of an all-fish gene cassette for gene transfer in aquaculture. Mol. Mar. Biol. Biotechnol. 1 (4-5) : 290-300. Ewart, K. V., Lin, Q. and Hew, C. L.1999. Review Structure, function and evolution of antifreeze proteins. Cell. Mole. Life Sci. 5 : 271-283. Feeney, R. E. and Yeh, Y. 1978. Antifreeze proteins from fish blood. Adv. Protein Chem. 32 : 192-282. Feeney, R. E. and Yeh, Y. 1993. Antifreeze protein : properties, mechanism of action and possible applications. Food Technol. 1 : 82-90. Feeney, R. E. and Yeh, Y. 1998. Antifreeze protein : current status and possible food uses. Trends Food Sci. Technol. 9 : 102-106. Fu, Y., Wang, Y. and Evans, S. M. 1998. Viral sequences enable efficient and tissue-specific expression of transgenes in Xenopus. Nat. Biotechnol. 16 : 253-257. Gong, Z., Yan, T., Liao, J., Lee, S. E., He, J. and Hew, C. L. 1997. Rapid identification and isolation of Zebrafish cDNA clones. Gene 201 : 87-98. Gordon, J. W., Santos, G. A., Plotkin, D. J., Barbuda, J. A. and Riddle, F. H. 1980. Genetic transformation of mouse embryos by microinjection of purified DNA. Proc. Natl. Acad. Sic. 77 : 7380-7384. Hagedorn, M. Hsu, E. Kleinhans, F. W. and Wildt, D. E. 1997. New approaches for studying the permeability of fish embryos : toward successful cryopreservation. Cryobiology 34 : 335-347. Harvey, B. 1983. Cooling of embryonic cells, isolated blastoderm and inact zebrafish Brachydanio rerio to -196?C. Cryobiology 20 : 440-447. Harvey, B., Kelley, R. N. and Ashwood-Smith, M. J. 1983. Permeability of inact and dechorionated zebrafish embryos to glycerol and dimethyl sulfoxide. Cryobiology 20 : 432-439. Her, G. M., Yeh, Y. H. and Wu, J. L. 2003. 435-bp liver regulatory sequence in the liver fatty acid binding protein (L-FABP) gene is sufficient to modulate liver regional expression in transgnic zebrafish. Dev. Dyn. 227 : 347-356. Hew, C. L., Poon, R., Xiong, F., Gauthier, S., Shears, M.A., King, M. J., Davies, P. L., and Fletcher, G. L. 1999. Liver-specific and seasonal expression of transgenic Atlantic salmon harboring the winter flounder antifreeze protein gene. Transgenic Res. 8 : 405-414. Houston, M. E. Jr., Chao, H., Hodges, R. S., Sykes, B. D., Kay, C. M., Sonnichsen, F. D. 1998. Binding of an oligopeptide to a specific plane of ice. J. Biol. Chem. 273 : 11714-11718. Inoue, K. 1992. Expression of reporter genes introduced by microinjection and electroporation in fish embryos and fly. Biotechnology 1 : 266-270. Jaenisch, R. 1988. Transgenic animals. Science 240 : 1468-1474. Janik, M., Kleinhans, F. W. and Hagedorn, M. 2000. Overcoming a permeability barrier by microinjection cryoprotectants into zebrafish embryos (Brachydanio rerio). Cryobiology 41 : 25-34. Lele, Z. and Krone, P. H. 1996. The zebrafish as a model system in developmental, toxicological and transgenic research. Biotech. Adv. 14 : 57-72. Li, X. M., Trinh, K. Y., Hew, C. L., Buettner, B., Baenziger, J. and Davies, P. L. 1985. Structure of an antifreeze polypeptide and its precursors from the ocean pout. J. Biol. Chem. 260 : 12904-12909. Lin, T. P. 1966. Microinjection of mouse eggs. Science 151 : 707-714. Liu, X. H., Zhang, T. and Rawson, D. M. 1999. The effect of partial removal of yolk on the chilling sensitivity of zebrafish embryos. Cryobiology 39: 236-242. Long, O., Meng, H., Jessen, J. R., Farrell, M. J.

and Lin, S. 1997. GATA-1 expression pattern can be recapitulated in living transgenic zebrafish using GFP reporter gene. Development 124 : 4105-4111. Lovelock, J. E. 1954. The protective action of neutral solutions against haemolysis by freezing and thawing. Biochem. J. 56 :265. Maclean, N., Penman, D. and Zhu, Z. 1987. Introduction of novel genes into fish. Biotechnology 5 : 257-261. Marray, J. D., Nancarrow, C. D., Marshall, J. T., Hazelton, I. G. and Ward, K. A. 1989. Production of transgenic merino sheep by microinjecton of ovine metallothionein ovine growth hormone fusion genes. Anal. Sci. 1 : 147-155. Mazur, P. 1977. The role of intracellular freezing in the death of cells cooled at supraoptimal rates. Cryobiology 14 : 251. Ng, N. F. L. and Hew, C. L. 1992. Structure of an antifreeze polypeptide from the sea raven: disulphide bonds and similarity to lectin-binding protein. J. Biol. Chem. 267 : 16069-16075. Onishi, A., Iwamoto, M., Akita, T.,Mikawa, S.,Awata, T., Hanada, H. and Perry. A. C. 2000. Pig cloning by microinjection of fetal fibroblast nuclei. Science 289 : 1188-1190. Paiement, J., Dominguez, J. M., Mcleese, J., Bernier, J., Roy, L. and Bergeron, M. 1990. Morphogenesis of endoplasmic reticulum in Xenopus oocytes after microinjection of rat liver smooth microsomes. Am. J. Anat. 187 : 183-192. Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G, Birnberg, N. C. and Evans, R. M. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion gene. Nature 300:611-615. Payne, S. R. and Young, O. A. 1995. Effect of preslaughter administration of antifreeze proteins on frozen meat quality. Meat Sci. 41:147-155. Peterson, K. R., Clegg, C. H., Huxley, C. Josephson, B. M., Haugen, H. S., Furukawa and Stamatoyannopoulos, G. 1993. Transgenic mice containing a 248-kb yeast artificial chromosome carrying the human beta-globin display proper developmental control of human globin genes. Proc. Natl. Acad. Sci. 90 : 7593-7597. Polge, C., Smith, A. U. and Parkes, A. S. 1949. Survival of spermatozoa after vitrification and dehydration at low temperature. Nature 164 : 166. Rall, W. F. 1987. Factors affecting the survival of vitrified embryos. Cryobiology 24 : 387-402. Rall, W. F. and Fahy, G. M. 1985. Ice free cryopreservation of mouse embryos at -196?C by vitrification. Nature 313 : 573-575. Rubinsky, B., Arav, A. and DeVries, A. L. 1991. Cryopreservation of oocytes using directional solidification and antifreeze glycoproteins. Cryo-Letters 12 : 93-106. Rubinsky, B., Mattioli, M., Arav, A., Barboni, B., and Fletcher, G. L. 1992. Inhibition of Ca2＋ and K＋ currents by antifreeze proteins. Am. J Physiol. 262 : 542-545. Scott, G. K., Hew, C. L. and Davies, P. L. 1985. Antifreeze protein genes are tandemly linked and clustered in the genome of the winter flounder. Proc. Natl. Acad. Sci. 82 : 2613-2617. Shears, M. A., Fletcher, G. L., Hew, C. L., Gauthier, S. and Davies, P. L. 1991. Transfer, expression and stable inheritance of antifreeze protein genes in Atlantic salmon (Salmon salar). Mol. Mar. Biol. Biotech. 1 : 58-63. Shevach, F., Linda, C. G. and Emmet, J. L. 1988. Cryopreservation of embryos and ova. Fertil. Steril. 49 : 743. Soltys, K. A., Batta, A. K. and Baburao, K. 2001. Successful nonfreezing, subzero preservation of rat liver with 2.3-butanediol and type Ⅰ antifreeze protein. J. Surg. Res. 96 : 30-34. Strauass, W. M., Dausman, J., Beard, C., Johnson, C., Lawrence, J. B. and Jaenish, R. 1993. Germ line transmission of a yeast artificial chromosome spanning the murine alpha 1 ( Ⅰ ) collagen locus. Science 259 : 1904-1907. Sun, A. Y. and Li, D. X. 1990. Ventricular arrhythmia evoked by microinjection of picrotoxin into brain areas in rabbits. Zhongguo Yao Li Xue Bao 11 : 296-299. Tsai, H., Wang, S. H., Inoue, K., Takagi, S., Kimura, M., Wakamatsu, Y. and Ozato, K. 1995. Initiation of the transgenic lacZ gene expression in medaka (Oryzias latipes) embryos. Mol. Mar. Biol. Biotechnol. 4 : 1-9. Wang, R., Zhang, P., Gong, Z. and Hew, C. L. 1995. Expression of the antifreeze protein gene in transgenic goldfish (Carassius auratus) and its implication in cold adaptation. Mol. Mar. Biol. Biotechnol. 4(1) : 20-26. Warren, C. J., Muler, G. M. and McKown. R. L. 1992. Ice crystal growth suppression polypeptides and methods of preparation. US Patant 5 : 118, 792. Whittingham, D. G., Leibo, S. P. and Mazur, P. 1972. Survival of mouse embryos frozen to -196?C and -296?C. Science 178 : 411-414. Willadsen, S. M., Polge, C., Rowson, L. E. A. and Moore, R. M. 1974 .Preservation of sheep embryos in liquid nitrogen. Cryobiology 11 : 560. Wilmut, I. and Rowson, L. E. A. 1973. Experiments on the low-temperature preservation of cow embryos. Vet. Rec. 93 : 686-690. Winkler, C., Vielkind, J. R. and Schartl, M. 1991. Transient expression of foreign DNA during embryonic and larval development of the medaka fish (Orizias latipes). Mol. Gen. Genet. 226 : 129-140. Yamamoto, Y., Oguri, N., Tsutsumi, Y. and Hachinohe, Y. 1982. Experiments in the freezing and storage of equine embryos. J. Reprod. Fert. Suppl. 32 : 399-403. Yamashita, Y., Miura, R., Takemoto, Y., Tsuda, S., Kawahara, H. And Obata, H. 2003. Type Ⅱ antifreeze protein from a mid-latitude freshwater fish, Japanese smelt (Hypomesus nipponensis). Biosci Biotechnol. Biochem. 67 : 461-466. Yang, D. S., Sax M., Chakrabatty, A. and Hew, C. L. 1988. Crystal structure of an antifreeze polypeptide and its mechanistic implications. Nature 333 : 232-237. Yeh, Y. and Feeney, R. E. 1996. Antifreeze proteins:structures and mechanisms of functions. Chem. Rev. 96 : 601-917. Zhang, T., Rawson, D. M. and Morris, G. J. 1993. Cryopreservation of pre-hatch of zebrafish. Aqua. Living Resour. 6 : 145-153. Zhang, T. and Rawson, D. M. 1995. Studies on chilling sensitivity of zebrafish (Brachydanio rerio) embryos. Cryobiology 32 : 239-246. Zhang, T. and Rawson, D. M. 1996. Feasiblity studies on vitrification of intact Zebrafish (Brachydanio rerio) embryos. Cryobiology 33 : 1-13. Zhang, T., Liu, X. H. and Rawsun, D. M. 2003. Effects of methanol and developmental arrest on chilling injury in zebrafish (Danio rerio) embryos. Theriogenology 59 : 1545-1556.