乳鐵蛋白應用於蝦類抗白點症病毒之研究

詹雲翔、張雲祥

E-mail: 9602311@mail.dyu.edu.tw

摘 要

白點症病毒(white spot syndrome virus, WSSV)是甲殼類生物的一個重要病毒，對養殖蝦有很高的致死率，目前尚無報導指出有任何一種蝦類對白點症病毒有抵抗力。乳鐵蛋白(lactoferrin, LF)是一種人類及動物體內普遍存在的攜鐵醣蛋白，屬於運鐵蛋白家族(transferrin family)成員之一，具有調節人及動物生理、抑制細菌和病毒繁殖、促進淋巴細胞的分化、調節巨嗜細胞(macrophage)、顆粒細胞(granulocyte)的增生以及在腸道中幫助鐵離子之運送及吸收等功能。本研究目的在探討牛乳鐵蛋白是否可以增進蝦類的抗白點症病毒感染能力並藉由草蝦之免疫指標酚氧化酵素（phenoloxidase activity）、超氧離子（superoxide anion, O2－）、超氧歧化酵素活性（superoxide dismutase activity, SOD）及草蝦免疫相關基因表現之分析探討其可能之抗病毒機制。以0.01、0.04及0.16 mg/g蝦體重之三種牛乳鐵蛋白濃度進行抗白點症病毒實驗，結果顯示牛乳鐵蛋白可以減緩蝦子的死亡率，其中以0.04 mg/g蝦體重的作用濃度最好，可使死亡率降低至59 %。以0.01及0.04 mg/g蝦體重之牛乳鐵蛋白注射草蝦，顯示蝦體內之酚氧化酵素、超氧離子和超氧歧化酵素活性均比控制組草蝦均出現顯著差異(P＜0.05)，酚氧化酵素活性於第2、12及24小時出現顯著差異(P＜0.05)，作用濃度以0.01 mg/g蝦體重之濃度效果最好；超氧離子於第48、72及96小時出現顯著差異(P＜0.05)，作用濃度以0.04 mg/g 蝦體重之濃度效果最好；超氧歧化酵素於第36、48及60小時出現顯著差異(P＜0.05)，作用濃度以0.04 mg/g蝦體重之濃度效果最好。在免疫基因表現之分析中，以0.01及0.04 mg/g蝦體重之牛乳鐵蛋白注射草蝦，結果顯示牛乳鐵蛋白能誘發一些草蝦免疫相關基因表現，作用濃度以0.04 mg/g蝦體重之濃度所誘發表現之基因數目最多。所以牛乳鐵蛋白有可能可以藉由提升草蝦之免疫能力增強其抗白點症病毒之感染。

關鍵詞 : 草蝦 ; 乳鐵蛋白 ; 白點症病毒

目錄

參考文獻

林婉曼。 1998。 草蝦超氧岐化酵素之研究:病毒感染、純化與生物特性之研究。國立台灣海洋大學水產生物技術研究所碩士學位論文。

宋延齡，黃志成，1999。應用免疫賦活劑以防治蝦類疾病。生物產業10(3):159-169。 陳秀男，黃世鈴，汪俊昇，王俊順，劉文御，郭光雄，1994。養殖蝦類白斑症及其防治對策。養魚世界，212:55-69。 陳威宇，2003。蝦白點症病毒感染後草蝦基因表現之分析。國立台灣大學動物學研究所碩士學位論文。 張正芳，2000。多醣類應用於強化草蝦抗弧菌與病毒之研究。國立中山大學海洋生物研究所博士學位論文。 郭光雄、宋延齡、陳秀男、廖一久、游祥平、謝大文、鍾忠勇、羅竹芳。1992 。蝦。黎明文化事業股份有限公司。台北。楊佳宏，2002。應用幾丁質及幾丁聚醣強化草蝦抵抗白點桿狀病毒及弧菌感染之研究。國立中山大學海洋生物研究所在職專班碩士學位論文。 Allen, R. C., Stjernholm, R. L., Steele, R. H. 1972. Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. Biochem. Biophys. Res. Commun. 47: 679-684. Andersen, J. H., Osbakk, S. A., Vorland, L. H., Traavik, T. and Gutteberg, T. J.2001. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Res. 51(2): 141-149. Arnold, D., Di-Biase, A. M., Marchetti, M., Pietrantoni, A., Valenti, P.,Seganti, L . and Superti, F. 2002. Antiadenovirus activity of milk proteins:lactoferrin prevents viral infection. Antiviral Res. 53(2): 153-158. Auffert, M. 1988. Bivalve hemocytes morphoiogy. In “Disease processes in marine bivalve mollusks” Am. Fish. Soc. Special Publication 18. By W.S. Fisher. (ed) Am. Fish. Soc., Washington. D.C. 169-177. Babior, B. M., Kipnes, R. S. and Curnutte, J. T. 1973. The production by leukocytes of superoxide, a potential bactericidal agent. J. Clin. Invest.52: 741-744. Barracco, M. A., Duvic, M. B. and Soderhall, K. 1991. The s-1,3-glucan binding protein from the crayfish Pacifastacus leniusculus when reacted with s-1,3-glucan, induces spreading and degranulation of crayfish granular cells. Cell Tissur Res. 266: 491-497. Babior, B. M. 1984. Oxidants from phagocytes: agent of defense and destruction. Blood. 959-966. Bauchau, A. G. 1981. Crustacean. In: Invertebrate blood cell. Ratcliffe, N. A.and Rowloy, A. F. (Eds) London: Academic Press. 387-417. Bayne, C. J., Moore, M. N., Carefoot, T. H. and Thompson, R. J. 1979.Hemolymph functions in Mytilus californianus. The cytochemistry of hemocytes and their responses to foreign implants and hemolymph factors in phagocytosis. J. Invertebr. Pathol. 34: 1-20. Baveye, S., Elass, E., Mazurier, J., Spik, G., and Legrand, D. 1999. Lactoferrin:a multifunctional glycoprotein involved in the modulation of the inflammatory process. Clin. Chem. Lab. Med. 37(3): 281-286. Berkhout, B., van-Wamel, J. L ., Beljaars, L., Meijer, D. K., Visser, S. and Floris, R. 2002. Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. Antiviral Res. 55(2): 341-355. Bell, K. L. and Smith, V. J. 1993. In vitro superoxide production by hyaline cells of the shore crab Carcinus maenas (L.). Dev. Comp. Immunol. 17: 211-219. Beauchamp, J. and Fridovich, I. 1971. Superoxide dismutase; improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287. Cardenas, W. and Dankert, J. R. 1997. Phenoloxidase specific activity in the red swamp crayfish Procambarus clarkii. Fish & Shellfish Immunol., 7: 283-295. Cerenius, L. and Soderhall, K. 1995. Crustacean immunity and complement: a premature comparison. Am. Zool. 35: 60-67. Chen, S. N., Chang, P.S., Kou, G.H. 1989. Observation on pathogenicity and epizootiology of Penaeus monodon baculovirus in cultured shrimp in Taiwan. Fish Pathol 24: 127-130. Chou, H.Y., Huang, C.Y., Wang, C.H., Chiang, H.C., Lo, C,F. 1995. Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeidshrimp in Taiwan. Dis Aquat Org 23: 165-173. Cheng, T. C. 1975. Functional morphology and biochemistry of molluscan phagocytes. Annals of the New York Academy of Sciences. 266: 343-379. Destoumieux, D., Saulnier, D., Garnier, J., Jouffrey, C., Bulet, P. and Bachere,E. 2001. Crustacean immunity-antfungal peptides are generated from the c-terminus of shrimp hemocyanin in response to microbial challenge. J.Biol. Chem. 276: 47070-47077. Destoumieux, D., Bulet, P., Strub, J. M., Vandorsselaer, A., Rodriguez, J. and Bachere, E. 1999. Recombinant expression and range of activity of penaeidins, antimicrobial peptides from penaeid shrimp. Eur. J. Biochem. 266: 335-346. Di Guilio, R. T., Washburn, P. C., Wenning, R. J., Winston, G. W. and Jewwll,C. S. 1989. Biochemical responses in aquatic animals: a review of determinant of oxidative stress. Environ, Toxicol. Chem. 8: 1103-1123. Ey, P. L. 1991. Phagocytosis in crustacea : The role of opsonins. In : Gupta, A.P. (Ed.), Immunology of insects and other arthropods. CRC press, Boca Raton, FL. 201-235. Farnaud, S. and Evans, R.W. 2003. Lactoferrin-a multifunctional protein with antimicrobial properties. Molecular immunology. 40: 395-405 Frisovich, I. 1986. Superoxide dismutase. Adv. Enzymol. 58: 61-79. Goldenberg, P. Z., Huebner, E. and Greenberg, A. H. 1984. Activation of lobster hemocytes for phagocytosis. J. Invertebr. Pathol. 43: 77-88. Fridovich, I. 1985. Superoxide radical and superoxide dismutases in E. coli.Fed. and Amer. Soc. Exp. Bio. 21-26. Gollas-Galvan, T., Hernandez-Lopez, J. and Vargas-Albores, F. 1999. Prophenoloxidase from brown shrimp Penaeus californiensis hemocytes.Comp. Biochem. Physiol. 122B: 77-82. Goldenberg, P. Z., Huebner, E. and Greenberg, A. H. 1984. Activation of lobster hemocytes for phagocytosis. J. Invertebr. Pathol. 43: 77-88. Hall, J. L. and Rowlands, D. T., 1974. Heterogeneity of lobster agglutinins. II.Specificity of agglutinin-erythrocyte binding. Biochemistry 13: 828-832. Holmblad, T. and Soderhall, K. 1999. Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity.Aquaculture 172: 111-123. Hose, J. E., Martin, G. C. and Gerard, A. S. 1990. A decapod hemocyte classification scheme integrating morphology, cytochemistry, and function. Biol. Bull. 178: 33-45. Iwasa, M., Kaito, M., Ikoma, J., Takeo, M., Imoto, I., Adachi, Y., Yamauchi,K., Koizumi, R. and Teraguchi, S. 2002. Lactoferrin inhibits hepatitis C virus viremia in chronic hepatitis C patients with high viral loads and HCV genotype 1b. Am. J. Gastroenterol. 97(3): 766-767. Johansson, M. W. and Soderhall, K. 1989. Cellular immunity in crustaceans and the proPOsystem. Parasit. Today. 5: 171-176. Johansson, M. W. and Soderhall, K. 1985. Excyrosis of the prophenoloxidase activation system from crayfish hemocytes. J. Comp. Physiol. 156: 175-181. Kakuta, I., Kurokura, H. 1995. Defensive effect of orally administered bovine lactoferrin against Cryptocaryon irritans infection of red sea bream. Fish Pathol., 30: 289-290. Le-Moullac, G., Le-Groumellec, M., Ansquer, D., Froissard, S., Levy, P. and Aquacop, F. 1997. Haemotological and phenoloidase activity changes in the shrimp Penaeus stylirostris in relation with the moult cycle:protection against vibriosis. Fish Shellf. Immunol. 7: 227-234. Levay, P. F. and Viljoen, M. 1995. Lactoferrin: a general review.Haematologica. 80(3): 252-267. Liao, I.C., Huang, T.L., and Kastsutani, K. 1969. A preliminary report on artificial. Propagation of Penaeus monodon Fabricius, JCCR Fish. Ser., 8: 67-71 Lightner, D.V., Redman, R.M., Poulos, B.T., Nunan, L.M., Mari, J.L., and Hasson, K.W.

1997. Risk of spread of penaeid shrimp viruses in the Americas by international movement of live and frozen shrimp. Rev Sci Tech Off Int Epiz. 16: 140-160. Lin, T. Y., Chu, C. and Chiu, C. H. 2002a. Lactoferrin inhibits enterovirus 71 infection of human embryonal rhabdomyosarcoma cells in vitro. J. Infect.Dis. 186(8): 1161-1164. Lo, C. F., Ho, C. H., Peng, S. E., Chen, C. H., Hsu, H. E., Chiu, Y. L.,Chang, C. F., Liu, K. F., Su, M. S., Wang, C. H. and Kou, G. H.1996a. White spot syndrome associated virus (WSSV) detected in cultured and captured shrimp, crabs and other arthropods. Dis.Aquat. Org., 27: 215-225. Lo, C. F., Leu, J. H., Ho, C. H., Chen, C. H., Peng, S. E., Chen, Y. T.,Chou, C. M., Yeh, P. Y., Huang, C. J., Chou, H. Y., Wang, C. H. and Kou, G. H. 1996b. Detection of baculovirus associated with white spot syndrome (WSSV) in penaeid shrimps using polymerase chain reaction. Dis. Aquat. Org., 25: 133-141. Lo, C. F., Kou, G. H. 1998.Virus associated white spot syndrome of shrimp in Taiwan: a review. Fish Pathol 33: 365-371. Lo, C. F., Hsu, H. C., Tsai , M. F., Ho, C. H., Peng, S. E., Kou, G. H., Lightner, D.V. 1999. Specific genomic DNA fragment analysis of different geographical clinical samples of shrimp white spot syndrome virus. Dis Aquat Org 35:175-185. Liao, I.C., Huang, T.L., Katsutain, K. 1969. A preliminary report on artificialpropagation of Penaeus monodon Fabricius. Reports of Fish Culture mResearch Supported by Rockfeller Foundation, JCRR Fish Ser 8: 67-71. Liao, I.C. 1989a. Penaeus monodon culture in Taiwan: Through two decades ofgrowth. Int J Aquacult Fish Technol 1:16-24. Liao, I.C. 1989b. Taiwanese shrimp culture: A molting industry. In: Chauvun K,Meneses P, Chauvin W, Cuccia A, eds. Proc Shrimp World IV (The Fourth Shrimp World Marketing Conference), Shrimp World Incorporated, New Orleans, Louisiana, U.S.A., 55-83. Mari, J., Bonami, J. R. and Lightner, D. V. 1993. Partial cloning of infectious hypodermal and haematopoietic necrosis virus, an unusual parvovirus pathogenic of penaeid shrimps; diagnosis of the disease using a specific probes. J. Gen. Virol. 74: 2637-2643. Mix, M. C. and Sparks, A. K. 1980. Hemocyte classification and differential counts in the Dungeness crab, Cancer magister. J. Invert. Pathol. 35:134-143. Moriuchi, M. and Moriuchi, H. 2001. A milk protein lactoferrin enhances human T cell leukemia virus type I and suppresses HIV-1 infection. J.Immunol. 166(6): 4231-4236. Moore, M. N. and Eble, A. F. 1977. The cytology and cytochemistry of the hemocytes of Mytilus edulis and their responses to experimentally injected carbon particles. J. Invert. Pathol. 29:248-256. Mori, K. and Stewart, J. E. 1978. Natural and induced bactericidal activity of the hepatopancreas of the American lobster, Homarus americanus. J.Invert. Pathol. 32: 171-176. Momoyama, K., Hiraoka, M., Nakano, H., Koube, H., Inouye, K., Oseko, N. 1994. Mass mortalities of cultured kuruma shrimp, Penaeus japonicus, in Japan in 1993: Histopathological study. Fish Pathol 29: 141-148. Murphy, M. E., Kariwa, H., Mizutani, T., Tanabe, H., Yoshimatsu, K., Arikawa, J. and Takashima, I. 2001. Characterization of in vitro and in vivo antiviral activity of lactoferrin and ribavirin upon hantavirus. Public Health. 63: 637-645. Nadala, E. C. B., Tapay, L,M., Loh, P. C. 1997. Yellow-head virus: a rhabdovirus-like pathogen of penaeid shrimp. Dis Aquat Org 31: 141-146. Nappi, A. J. and Vass, E. 1993. Melanogenesis and the generation of cytotoxic molecules during insect cellular immune reactions. Pigment. Cell. Res.171: 263-273. Ng, T. B., Lam, T. L., Au, T. K., Ye, X. Y. and Wan, C. C. 2001. Inhibition of human immunodeficiency virus type 1 reverse transcriptase, protease and integrase by bovine milk proteins. Life Sci. 69(19): 2217-2223. Nozaki, A., Tanaka, K., Naganuma, A. and Kato, N. 2002. Recent advances of basic research and clinical application of lactoferrin as an antiviral reagent against chronic hepatitis C. Nippon. Rinsho. 60(4): 819-829. Persson, M., Vey, A. and Soderhall, K. 1987. Encapsulation of foreign particles in vitro by separated blood cells from crayfish, Astacus leptodactylus. Cell Tissue Res. 247: 409-415. Perazzolo, L. M. and Barracco, M. A. 1997. The prophenoloxidase activating system of the shrimp, Penaeus paulensis and associated factors. Dev.Comp. Immunol. 21: 385 – 395. Perazzolo, L. M. and Barracco, M. A. 1997. The prophenoloxidase activating system of the shrimp, Penaeus paulensis and associated factors. Dev.Comp. Immunol. 21: 385 – 395. Ratcliffe, N. A., Rowley, A. F., Fitzgerald, S. N. and Rhodes, C. P. 1985 Invertebrate immunity-Basic concepts and recent advances. Int. Rev. Cytol., 97: 183-349. Sakai, M., Otubo, T., Atsuta, S., Kobayshi, M., 1993. Enhancement of resistance to bacterial infection in rainbow trout, Oncorhynchus mykiss (Walbaum) by oral administration of bovine lactoferrin. Fish Dis. 16: 239-247. Sanchez, L., Calvo,M. and Brock, .J. H. 1992. Biological role of lactoferrin.Arch. Dis. Child. 67(5): 657-661. Secombes, C. J. 1990. Isolation of salmonid macrophages and analysis of their killing activity. In Techniques in Fish Immunology (Stolen, J. S., Fletcher, T. C., Anderson, D. P., Roberson, B. S. and Van Muiswinkel, W. B. eds.). pp,. Fair Haven, NJ: SOS Publications. 137-154. Shau, H., Kim, A. and Golub, S. H.1992. Modulation of natural killer and lymphokine activated killer cell cytotoxicity by lactoferrin. J. Leukoc. Biol. 51(4): 343-349. Smith, V. J. and Soderhall, K. 1983. s-1,3-glucan activation of crustacean hemocytes in vitro and in vivo. Biol. Bull. 164: 299-314. Song, Y. L. and Hsieh, Y. T. 1994. Immunostimulation of tiger shrimp (Penaeus monodon) hemocytes for generation of microbicidal substances:analysis of reactive oxygen species. Dev. Comp. Immunol. 18: 201-209. Soderhall, K. and Cerenius, L. 1998. Role of prophenoloxidase activating system in invertebrate immunity. Curr. Opin. Immunol. 10: 23-28. Soderhall, K., Smith, V. J. and Johansson, M. W. 1986. Excytosis and uptake of bacteria by isolated hemocyte populations of two crustacean: Evidance for cellular cooperation in the defense reactions of arthropods.Cell Tissue Res. 245: 43-49. Soederhall, K. and Smith, V. J. 1983. Separation of the hemocyte populationsof Carcinus maenas and other marine decapods [J]. Dev. Comp. Immune.7: 229-239. Soderhall, K. 1982. Prophenoloxidase activating system and melanization-A recognition mechanism of arthropods. A review. Dev. Comp. Immunol. 6: 601-611. Soderhall, K. and Cerenius, L. 1992. Crustacean immunity. Ann. Rev. Fish Dis. 3-23. Stewart, J. E. and Foley, E. M. 1969. A preipitin reaction of the hemolymph of the lobster Homarus americanus. J. Fish Res. Bd. Can. 26: 1392-1397. Sritunyalucksana, K., Sithisarn, P., Withayachumnarnkul, B. and Flegel, T.W.1999. A preliminary study on activation of agglutinin and antibacterial activity in hemolymph of the black tiger prawn, Penaeus monodon. Fish Shellf. Immunol. 9: 21-30. Srimal, S., Dorai, D. T., Somasundaran, M., Bachhawat, B. K. and Miyata, T. 1985. A new hemagglutinin from the amoebocytes of the horseshoe crab Carcinoscorpius rotundicauda. Purification and role in cell aggregtion,Biochem. J. 230-321. Superti, F., Siciliano, R., Rega, B., Giansanti, F., Valenti, P. and Antonini, G. 2001. Involvement of bovine lactoferrin metal saturation, sialic acid and protein fragments in the inhibition of rotavirus infection. Biochim Biophys Acta. 528(2-3): 107-115.

Sung, H. H., Yang, Y. L. and Song, Y. L. 1996. Enhancement of microbicidal activity in tiger shrimp (Penaeus monodon) via immunostimulation. J.Crust. Biol. 16279-16285. Tsai, M.F., Kou, G. H., Liu, H. C., Liu, K.F., Chang, C.F., Peng, S. E., Hsu, H. C., Wang, C. H., Lo, C.F. 1999. Long-term presence of white spot syndrome virus (WSSV) in a cultivated shrimp population without disease outbreaks. Dis Aquat Org 38: 107-114. Van der Strate, B. W., Beljaars, L., Molema, G., Harmsen, M. C. and Meijer, D.K. 2001. Antiviral activities of lactoferrin. Antiviral. Res. 52(3): 225-239. Valimaa, H., Waris, M., Hukkanen, V., Blankenvoorde, M. F., Nieuw Amerongen, A. V. and Tenovuo, J. 2002. Salivary defense factors in herpes simplex virus infection. J. Dent. Res. 81(6): 416-421. Vargas-Albores, F., Guzman, M. A. and Ochoa, J. L. A. 1993. An anticoagulant solution for hemolymph collection and prophenoloxidase studies of penaeid shrimp Penaeus californiensis . Comp. Biochem.Physiol. 106A: 299-303. Vargas-Albores, F., Jimenez-Vega, F. and Soderhall, K. 1996. A plasma protein isolated from brown shrimp Penaeus californiensis which enhances the activation of prophenoloxidase system by s-1,3-glucan.Dev. Comp. Immunol. 20: 299-306. Winston, G. W., Moore, M. N., Kirchin, M. A. and Soverchia, C. 1996.Production of reactive oxygen species by hemocytes from the marine mussel, Mytilus edulis: lysosomal localization and effect of xenobiotics.Comp. Biochem. Physiol. 113(2): 221-229. Zhang, P., Sawicki, V., Lewis, A., Hanson, L., Nuijens, J. H. and Neville, M. C. 2001. Human lactoferrin in the milk of transgenic mice increases intestinal growth in ten-day-old suckling neonates. Adv. Exp. Med. Biol.501: 107-113.