ELSEVIER

Fish & Shellfish Immunology 20 (2006) 474–481

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Purification and characterization of α_2-macroglobulin from grass carp *Ctenopharyngodon idellus*: Cloning a segment of the corresponding gene

F.L. Li, C.P. Lu*

Key Laboratory of Animal Disease Diagnostic and Immunology Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, China

Received 1 April 2005; revised 12 May 2005; accepted 16 June 2005
Available online 31 August 2005

Abstract

The plasma protein α_2-macroglobulin (α_2M) was purified by gel filtration and anion-exchange chromatography from grass carp plasma. The α_2M consists of two different subunits of molecular weight 95 kDa and 80 kDa, respectively. The characteristics of grass carp α_2M are similar to mammalian α_2M, in that grass carp α_2M exists in two forms: a fast-form and a slow-form. The former is complexed with protease. The sequence of grass carp α_2M-conserved region and a region containing the bait region was determined by sequence analysis using polymerase chain reaction (PCR). The deduced amino acid sequence of the conserved region is similar to the α_2M sequence of common carp, however, the bait region amino acid sequence is dramatically distinct from that of common carp. This may partially explain the differential ability of α_2M of different species to inhibit different proteases. The α_2M was able to inhibit *Aeromonas hydrophila* extracellular protease (AhECPase) and thus may play a role in resistance to infection by this pathogen.

Keywords: Grass carp; *Ctenopharyngodon idellus*; α_2M; Purification; Amplification; Bait region; Sequence analysis

1. Introduction

The α_2M protein is a large glycoprotein found both in vertebrates [1] and in invertebrates [2–4]. It can help to remove endogenous proteases and various exogenous proteases, such as those secreted by invading bacteria [2–4].

* Corresponding author. Tel./fax: +86 25 84396517.
E-mail address: lucp@njau.edu.cn (C.P. Lu).

1050-4648/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2005.06.006

In an experiment to develop an infectious disease model, we discovered that grass carp infected with *Aeromonas hydrophila* (Ah) experience lower mortality in comparison to other species of fish [5,6]. We subsequently investigated the biochemical basis of this peculiar resistance to infection [6]. We found that there was no correlation between the natural anti-Ah immunoglobulin titer of grass carp and their ability to resist infectious challenge. This suggested that some non-specific immune factor(s) present in grass carp might be responsible, such as α_2M. In the present work, we showed that purified α_2M from the plasma of grass carp was able to inhibit an extracellular protease of *A. hydrophila*.

We cloned the genes of grass carp α_2M, and analyzed and compared their sequences with those of other animal species in an attempt to further understand the nature of the resistance of different types of fish against Ah.

2. Materials and methods

2.1. Materials

Grass carp weighing 1–1.5 kg were purchased from a local market. *A. hydrophila* J-1 was obtained from our laboratory [5]; Moloney murine leukemia virus reverse transcriptase (M-MLV) was purchased from the Promega Company; a total RNA extraction kit was from the Invitrogen Company; the Taq enzyme came from the Takara Company; the soya-bean trypsin inhibitor and rabbit anti-human α_2M serum were from the Sigma Chemical Company; and human α_2M was a gift from the Nanjing Military Medicine Institute in China.

2.2. Preparation of *A. hydrophila* extracellular protease (AhECPase)

A. hydrophila J-1 was grown at 37 °C for 24 h [5] and the extracellular protease (AhECPase) was contained in the crude lyophilized material obtained from the medium supernatant after precipitation with 70% $(NH_4)_2SO_4$ solution. The products were dissolved in 50 mM Tris–HCl (pH 7.8) at 4 °C and frozen at −20 °C before use.

2.3. Purification of α_2M from grass carp

Blood was taken from the caudal vein using a sterile syringe containing 3.8% precooled sodium citrate buffer as an anticoagulant. The blood was centrifuged at 1000 rpm for 15 min at 4 °C. The plasma was decanted into a plastic tube, and buffer containing 0.1% (w/v) soya-bean trypsin inhibitor, 10 mM EDTA and 0.02% NaN_3 was added. The plasma was then fractionated by polyethylene glycol gradient (PEG 6000) precipitation. The precipitate, collected by centrifugation in a 5–18% PEG gradient, was resuspended in 50 mM Tris–HCl buffer (pH 7.4). The crude product was centrifuged at 10 000 g for 5 min at 4 °C to remove precipitated proteins and the supernatant was then applied to a Sephacryl S200 high column equilibrated with 50 mM Tris–HCl buffer (pH 7.4). The flow-through, α_2M-containing fractions were pooled after confirming α_2M activity (see below). The pooled material was passed through a Hiprep16/10QXL column, previously equilibrated with 50 mM Tris–HCl buffer (pH 7.4). Bound protein was eluted with 50 mM Tris–HCl buffer (pH 7.0) containing 1 M NaCl in a linear gradient. The α_2M-containing fractions were pooled and analyzed for purity by SDS-PAGE.

2.4. Characterization of α_2M and assay of protease-inhibiting activity

When AhECPase is added to a well in an agarose gel plate containing casein for 3 h, a clear circle appears around the well. The presence of grass carp α_2M inhibits AhECPase activity and prevents the circle