JOURNAL OF PROTEOMICS 98 (2014) 218–232

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Characterization of carp seminal plasma proteome in relation to blood plasma

Mariola A. Dietrich[a,*], Georg J. Arnold[b], Joanna Nynca[a], Thomas Fröhlich[b], Kathrin Otte[b], Andrzej Ciereszko[a]

[a]Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Department of Gamete and Embryo Biology, Olsztyn, Poland
[b]Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-Universität, Munich, Germany

ARTICLE INFO

Article history:
Received 14 November 2013
Accepted 5 January 2014
Available online 13 January 2014

Keywords:
Carp
Seminal plasma
Blood plasma
Proteome
LC–MS/MS
2D-DIGE

ABSTRACT

The present study for the first time characterizes a diverse cohort of carp seminal and blood plasma proteins using the combination of protein fractionation by one-dimensional gel electrophoresis and high performance liquid chromatography electrospray ionization tandem mass spectrometry. Using this approach, we identified 137 proteins in carp seminal plasma and 88 proteins in carp blood plasma, most of which were newly identified in fish. Transferrin, serine proteinase inhibitors, apolipoproteins, complement C3 and Wap65 were present in high abundance in carp seminal plasma. In carp blood plasma, besides these proteins, immunoglobulins and macroglobulins were identified as major proteins. Comparative analysis of carp seminal and blood plasma proteome performed using 2D-DIGE revealed that in contrast to mammals the majority (1014 from 1240 spots) of carp seminal plasma proteins are blood proteins. Moreover, proteins more abundant in seminal plasma (99 from 1240 spots) were identified, including parvalbumin, isoforms of apolipoproteins, heat shock proteins, components of antioxidative system, matrix metalloproteinases, cathepsin D, enzymes of glycolysis and sperm structural proteins. These proteins are involved in the regulation of sperm motility, spermatogenesis, maintenance of sperm membrane lipid stability and antioxidant protection. This study enhances the basic knowledge concerning fish seminal plasma protein composition and their potential role in fish reproduction.

Biological significance
Proteins similar or identical to blood plasma components are important for male reproductive physiology. Comparative study of blood and seminal plasma is especially justified in fish. Using 2D-DIGE we indicated that, in contrast to mammals, in carp seminal plasma most proteins are common for blood and seminal plasma, which possibly is related to a lack of accessory glands in reproductive tract of most fish. The proteins present in higher abundance in seminal plasma can be related to physiology of fish male reproduction including regulation of sperm motility, spermatogenesis, maintenance of sperm surface composition and antioxidant protection. Application of proteomics analysis to identify carp seminal and blood plasma proteins significantly extends current knowledge regarding the composition of fish seminal and blood plasma proteins and their relationship to higher vertebrates. Moreover, proteomic profiling of carp seminal plasma appears to be helpful for further understanding of the role of fish seminal plasma proteins in male reproductive tract as well as for identification of novel biomarkers for sperm quality.

* *Corresponding author*. Tel.: +48 89 5393135.
E-mail address: m.dietrich@pan.olsztyn.pl (M.A. Dietrich).

1874-3919/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jprot.2014.01.005

1. Introduction

The general role of seminal plasma is to create an optimal environment for the storage of spermatozoa. The conditions of storage should protect sperm fertilizing ability and motility and maintain metabolism to prevent viability and energy resources for sperm activation [1]. Mammalian seminal plasma is a highly complex fluid that contains proteins secreted from testis, epididymis and male secretory sexual glands. In most teleost fishes, seminal plasma is a secretory product of only the testes and spermatic ducts [2,3]. Some components of seminal plasma originate from damaged spermatozoa and other somatic cells such as leukocytes, epithelial cells of the testes and spermatic ducts. Fish seminal plasma, in contrast to that of higher vertebrates, is characterized by a low protein concentration, containing mainly mineral compounds (sodium, potassium, calcium, magnesium) and low concentrations of other organic substances, such as hormones and pheromones, cholesterol, glycerol, vitamins, free amino acids, sugars, citric acid and lipids [1,4,5].

Characterization of protein components in fish seminal plasma has been performed via the isolation of single proteins and the identification using Edman sequencing. However, using this approach only major proteins of fish seminal plasma, visible after Coomasie Brilliant blue staining of one-dimensional polyacrylamide gels (1DE), were identified. For common carp seminal plasma, the following proteins were identified: serpin of α1-proteinase type [6], transferrin [7] and parvalbumin [8]. A few proteins were also identified in rainbow trout seminal plasma, such as α_1-antiproteinase, fetuin-B-like protein, lipocalin-type prostaglandin-D synthase and apolipoproteins [9–13]. Besides direct identification via sequencing, some information on seminal plasma proteins was indirectly obtained using measurements of enzymes or inhibitor activity. The presence of specific patterns of proteinase and serine proteinase inhibitors in cyprinid seminal plasma was described [14,15]. Calcium binding proteins were identified in salmonid and cyprinid seminal plasma [16]. However, in most cases, such proteins have not been specifically identified yet.

To study proteins on a large scale, the application of a proteomic approach is mandatory. Proteomic techniques have been widely used to identify seminal and blood plasma as well as sperm proteomes of higher vertebrates. However, limited information is available on the proteomes of fish sperm and seminal plasma. So far, two dimensional electrophoresis (2DE) and mass spectrometry have been applied to study differences in sperm protein profiles among sturgeon species [17,18], molecular mechanism determining the initiation of sea bream sperm motility [19], the effect of domestication on semen quality [20] and the effect of cryopreservation on carp sperm injury [21]. However, in these studies only a few protein spots with significantly altered expression were identified.

Proteins similar or identical to blood plasma components are important for male reproductive physiology. For example, in humans these proteins constitute about 10% of the seminal plasma proteins [22,23]. In mammals, many testicular fluid proteins are similar or identical to those found in blood, but many of them disappear progressively during epididymal transit which suggests their importance during spermatogenesis [24]. LaFlamme and Wolfner [25] indicated that many seminal plasma proteins contributing to seminal clotting and liquefaction were also expressed as blood plasma proteins. This suggests that similar physiological cascades operate both in seminal and blood plasma. Blood proteins can be either transported from blood or produced within the reproductive tract or both [23,26,27]. For example, 80% of transferrin is produced by Sertoli cells and 20% originates from blood [27]. In summary, the role of blood proteins in semen seems to be very important, but knowledge concerning this matter is still very limited.

A comparative study of blood and seminal plasma is especially justified in fish. The comparison of fish seminal and blood plasma performed using 1DE suggested that in seminal plasma most proteins are blood proteins [9,28]. For carp seminal plasma, using electrophoretic techniques (1DE and staining with antitrypsin and gelatinolytic proteases activity) four proteases and two proteinase inhibitors (inhibitor I and II) similar to blood were identified [14,15]. Additionally, α1-antiproteinases (inhibitor II) and transferrin (Tf) from carp blood and seminal plasma were found to be immunologically similar [6,7]. In contrast, few proteins including serine proteinase inhibitor III, two metalloproteinases and parvalbumin-like proteins were found only in carp seminal plasma [8]. Studies of the relationship between blood and seminal plasma proteins are prerequisite to understanding the origin of seminal plasma proteins. In addition, a higher abundance of proteins in seminal plasma may indicate their specific role in sperm physiology.

Carp (*Cyprinus carpio*) is an important aquaculture species worldwide and, besides being of commercial interest, it is also a research model organism within Teleostei. The aim of this study was to profile proteins from carp blood and seminal plasma and to compare their relative abundances in both sources. Two strategies were applied to achieve those goals: a combination of protein fractionation by 1D-SDS-PAGE and high performance liquid chromatography electrospray ionization tandem mass spectrometry (LC–MS/MS) for the proteomic characterization of carp seminal and blood plasma and 2D-DIGE (two dimensional difference gel electrophoresis) to determine and quantify proteins differently abundant in seminal plasma compared to blood plasma.

2. Material and methods

2.1. Semen and blood collection

Milt of common carp was obtained from fish maintained at the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences in Gołysz, Poland. Twenty-four hours before the collection of carp semen, the males were injected intradorsaly with Ovopel (one pellet containing of 18–20 μg of GnRH analog and 8–10 mg of metoclopramide per 1 kg of fish bw; Interfish Ltd, Hungary). The milt was obtained from 5 to 7 year old carp with an average body weight of 5–7 kg (n = 4). Milt samples were collected by gentle abdominal massage, taking care not to pollute them with blood, feces or urine. Polluted samples were discarded. Then milt was centrifuged at 3000 ×*g* for 30 min (4 °C), followed by centrifugation of