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International Journal of Biochemistry & Physiology
ISSN: 2577-4360

Fibrinogen in Hamster Cauda Sperm

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Review Article
Volume 8 Issue 2
Received Date: September 30, 2023
Published Date: October 21, 2023
DOI: 10.23880/ijbp-16000237

Abstract

Epididymis plays a vital role in promoting sperm maturation and maintaining sperm viability. It has been shown the presence of nonviable sperm in cauda epididymis. We previously identified a secretory protein (260/280KDa oligomers) of hamster cauda epididymal principal cells that binds to nonviable sperm. The 260/280KDa oligomers are composed of 64kDa FGL2 (fibrinogen-like protein-2) and 33kDa FGL1) (fibrinogen-like protein-1). In addition, we have demonstrated that FGL2 is a phospholipid-activated serine protease; the conversion of prothrombin to thrombin by FGL2 followed by the conversion of soluble fibrinogen to insoluble fibrin polymers by thrombin. In the present study, we have shown the presence of a 56kDa fibrinogen β in hamster cauda sperm. The potential role of fibrinogen in hamster physiology is being discussed.

Keywords: Hamster Sperm; Cauda Epididymis; Fibrinogen like Protein2; Fibrinogen; Fibrin

Abbreviations: DCF: Isolation of Death Cocoon Fraction; NGS: Normal Goat Serum; FGL2: Fibrinogen-Like Protein-2.

Introduction

Mammalian spermatozoa exiting the testis are functionally immature and require a series of post-testicular morphological and biochemical modifications in the epididymis to achieve forward motility and fertilizing capacity [1,2]. The luminal environment of the epididymis has a dual function. In the proximal epididymis, it promotes sperm maturation, and in the distal cauda epididymis, it maintains sperm viability during storage [1,3-8]. Although several studies proposed the putative roles of epididymal secretory proteins, the precise function of epididymal secretory proteins in sperm maturation and survival is not clearly understood. The presence of dead spermatozoa in the cauda epididymis region has been observed in many species; not all spermatozoa remain viable during passage through the epididymis; [9-11]. In the hamster, the nonviable sperm population increases along the length of the epididymis [11]. Nagdas SK, et al. [12] proposed another mechanism for recognizing and masking abnormal spermatozoa. Previously, we identified a hamster epididymal secretory protein of 64kDa (originally termed HEP64). Both luminal fluid and sperm-associated HEP64 are assembled into disulfide-linked oligomers of ~260kDa and ~280kDa. HEP64 is secreted by the principal cells of cauda epididymidis, and it binds explicitly to nonviable sperm and then polymerizes into a proteinaceous “death cocoon” that coats defective spermatozoa and sperm fragments [12]. HEP64 was cloned by expression screening of a hamster cauda epididymal cDNA library and identified as the hamster fibrinogen-like protein 2 (fgl2) [13], a fibrinogen-related family member containing a C-terminus fibrinogen-like domain [14] and an orthologue of mouse and human fgl2 [15-17]. Our previous

studies reveal that FGL2 and FGL1 proteins specifically bind defective spermatozoa and sperm fragments and then polymerize into a "death cocoon," segregating the defective sperm from the viable sperm populations [12,13,18]. Recently, we purified FGL2 from hamster cauda epididymal fluid towards homogeneity, and its prothrombinase catalytic activity was examined [19]. We have shown the downstream sequence of prothrombinase activity of FGL2; the conversion of prothrombin to thrombin by FGL2, followed by the conversion of soluble fibrinogen to insoluble fibrin polymers by thrombin. In the present study, we examined the presence of fibrinogen in hamster sperm.

Materials and Methods

Animals

Mature male golden hamsters were housed in Benedict College (Columbia, SC). Animal Care Facility on a 14L: 10D cycle and given free access to food and water. Care and use of animals conformed to NIH guidelines for humane animal care and use in research. All protocols were approved by the Institutional Animal Care and Use Committee and the University veterinarians who supervised animal care. Animals were sacrificed by CO_2 asphyxiation, and tissues were immediately removed for the protocols described below.

Preparation of Epididymal Sperm and Luminal Fluid Samples

The cauda epididymides were dissected and minced in calcium-free Tyrode solution at 37°C. The sperm suspension was centrifuged at 100 × g for 1 min to sediment tissue fragments, and the supernatant fluid was recentrifuged at 1,500 × g for 10 min at 4°C. Sperm pellets were used fractionation protocols described below.

Isolation of Death Cocoon Fraction (DCF)

Hamster cauda sperm death cocoon fractions were isolated following the method of Olson, et al. [13]. Briefly, sperm suspensions were prepared by mincing freshly dissected cauda epididymides in calcium-free Tyrode's solution containing 0.5 mM EGTA and 2 mM benzamidine. Aliquots of the sperm suspension (6 ml) were layered over an isotonic discontinuous Percoll gradient composed of 2 ml of 20% Percoll and 2 ml of 40% Percoll in Tyrode's solution. The gradients were centrifuged at 1,500 × g for 10 min. The particulate fraction that layered on top of the 20% Percoll was collected, diluted with ice-cold Tyrode's solution, and then sedimented at 100,000 × g for 60 min in a Beckman SW41 rotor. The isolated DCF fraction was suspended in TNI for the following biochemical analyses.

Gel Electrophoresis and Western Blotting

Polypeptides were separated by SDS-PAGE Laemmli [20] on a 12% separation gel prepared with a 30:0.8 acrylamide:bisacrylamide ratio. Western blots were prepared on polyvinylidene difluoride membranes for immunoblot analysis [21]. Proteins were estimated by the method of Bradford [22].

Immunoblots were blocked with TBS (0.15 M NaCl, 20 mM Tris-HCl Buffer, pH 7.5) containing 0.1% Tween 20 and 1% BSA and then incubated with immune serum (rabbit monoclonal fibrinogen beta chain antibody [abcam]) or nonimmune serum diluted in TBS containing 0.1% Tween 20 (TBS-TW) and 1% BSA followed by three washes in TBS-TW. For LICOR Odyssey CLx Imaging System IRDye ® 680RD, goat anti-mouse and goat anti-rabbit diluted in TBS-TW were used. Following several TBS-TW washes, immunoreactive bands were identified either by LICOR Odyssey CLx Imaging System.

Immunofluorescence Microscopy

Sperm suspension of cauda epididymis was fixed for 15–30 min at 4 °C with 4% formaldehyde, 0.1 M sodium phosphate buffer, pH 7.4 (PBS) and plated on poly-L-lysine-coated coverslips. After three rinses in PBS containing 0.05% Tween 20, nonspecific protein binding sites were blocked in PBS containing 1% normal goat serum, 1% normal donkey serum, and 2.5% BSA. After three rinses in PBS containing 1% normal goat serum (PBS-NGS), coverslips were incubated with equal dilutions of immune or nonimmune serum in PBS-NGS for 1 hr. and washed three times with PBS-NGS. Cells were then incubated with Cy3-conjugated goat anti-rabbit IgG (KPL Inc., Gaithersburg, MD) in PBS-NGS for 1 hr. Coverslips were washed with PBS and were examined by phase contrast and epifluorescence microscopy.

Results and Discussion

Western blot analysis of hamster cauda death cocoon fraction (lane 1), and cauda epididymal sperm (lane 2) stained with anti-fibrinogen beta chain antibody revealed the presence of a 56kDa fibrinogen β chain polypeptide in both fractions (Figure 1). Immunofluorescence localization of fibrinogen β chain of hamster cauda epididymal spermatozoa using anti-fibrinogen beta chain antibody demonstrated the presence of a strong signal to the equatorial segment of the sperm head, and a faint stain was present in the tail (Figure 2A). Sperm stained with nonimmune serum exhibited no fluorescence (Figure 2B). Our biochemical and cytochemical studies demonstrate the presence of a 56kDa fibrinogen β in hamster cauda sperm.

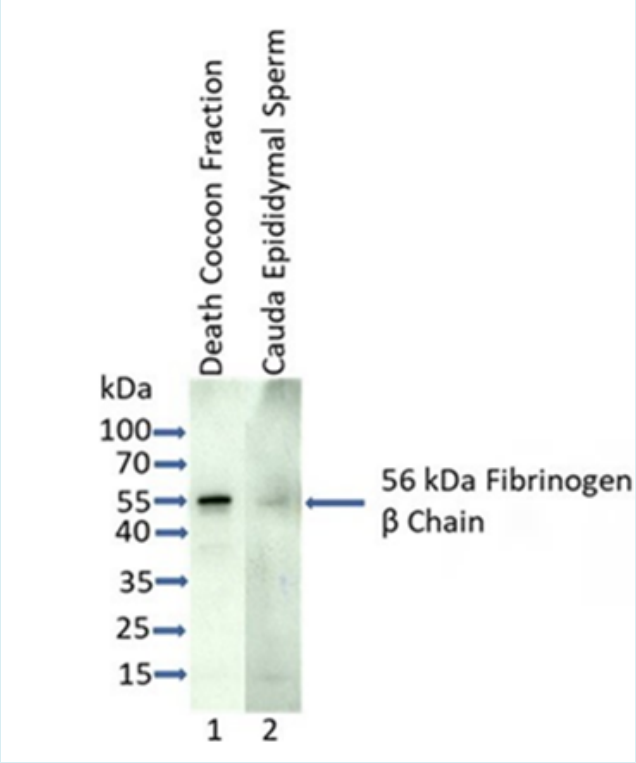

Figure 1: Immunoblot analysis of death cocoon fraction, and cauda epididymal sperm stained with anti-fibrinogen beta chain antibody revealed the presence of a 56kDa fibrinogen β chain polypeptide. Each lane contained 5µg protein.

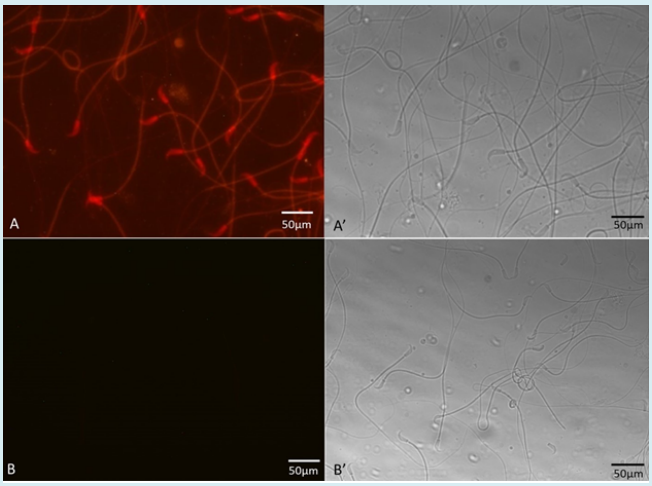

Figure 2: Fluorescence (A, B) and matched phase-contrast (A,' B') photomicrographs of cauda epididymal spermatozoa immunostained with anti- fibrinogen beta chain antibody (A) revealed intense staining to the equatorial segment of the sperm head and faint staining in the tail. Sperm stained with nonimmune serum showed no fluorescence (B). Bar = 50 µm.

Previously, we have shown the prothrombinase activity of hamster cauda epididymal FGL2 converting prothrombin to thrombin, and it is a lipid–activated serine protease. We have shown the presence of fibrinogen β chain in hamster cauda epididymal sperm and mostly localized in the equatorial segment of the sperm head (Figure 2). Several investigators reported the presence of fibrinogen gamma-B chains (a doublet of 50– 52 kDa) in bovine epididymal epithelium fluid [23] and seminal plasma [24] and proposed that fibrinogen gamma-B chains play a role in sperm protection in vivo [23]. It is also reported that fibrinogen-like substance in human seminal plasma plays an important role in the coagulation process in human semen [25]. Park, et al. [25] also suggested that certain steps of the coagulation process in human semen have the same process as the last step of the blood coagulation system. Several critical questions about the role of the fibrinogen β chain in hamster cauda sperm remain unresolved. It may be involved in membrane integrity, preventing premature acrosome reaction, or acting as anti-agglutination factors to regulate the eventual induction of capacitation and to extend sperm longevity. Future studies will address the role of fibrinogen in sperm physiology.

Conclusion

We have shown the presence of a 56kDa fibrinogen β in hamster cauda sperm and immunolocalization study reveals that fibrinogen β polypeptide is mostly present in the equatorial segment of the sperm head, and a weak staining was observed in the tail.

Acknowledgements: Supported by NIH grant 5SC3GM125488 (Subir K Nagdas) and GM 103499 (S. Raychoudhury)

Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contribution Statement: Each author has contributions to the article.

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