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Isolation and biochemical characteristics of a molecular form of epididymal acid phosphatase of boar seminal plasma

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Abstract

The fluid of boar epididymis is characterized by a high activity of acid phosphatase (AcP), which occurs in three molecular forms. An efficient procedure was developed for the purification of a molecular form of epididymal acid phosphatase from boar seminal plasma. We focused on the epididymal molecular form, which displayed the highest electrophoretic mobility. The purification procedure (dialysis, ion exchange chromatography, affinity chromatography and hydroxyapatite chromatography) used in this study gave more than 7000-fold purification of the enzyme with a yield of 50%. The purified enzyme was homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified molecular form of the enzyme is a thermostable 50 kDa glycoprotein, with a pI value of 7.1 and was highly resistant to inhibitors of acid phosphatase when *p*-nitrophenyl phosphate was used as the substrate. Hydrolysis of *p*-nitrophenyl phosphate by the purified enzyme was maximally active at pH of 4.3; however, high catalytic activity of the enzyme was within the pH range of 3.5–7.0. Kinetic analysis revealed that the purified enzyme exhibited affinity for phosphotyrosine ($K_m = 2.1 \times 10^{-3}$ M) and was inhibited, to some extent, by sodium orthovanadate, a phosphotyrosine phosphatase inhibitor. The N-terminal amino acid sequence of boar epididymal acid phosphatase is ELRFVTLVFR, which showed 90% homology with the sequence of human, mouse or rat prostatic acid phosphatase.

The purification procedure described allows the identification of the specific biochemical properties of a molecular form of epididymal acid phosphatase, which plays an important role in the boar epididymis.

Keywords: Boar; Acid phosphatase; Epididymis; Seminal plasma

1. Introduction

It has been known that the fluid of the epididymis is of primary interest because it controls or mediates epididymal function. The acquisition of sperm motility and fertilizing ability during epididymal transit has been associated with different biochemical changes in the male gamete. The epididymis synthesizes and secretes numerous proteins, which interact with the sperm plasma membrane [1,2]. Evidence has been shown that an array of specific glycoproteins, synthesized in the epididymis, are absorbed on the surface of the sperm plasma membrane [3–5]. The fluid of the epididymis also contains enzymatic proteins, such as lactate dehydrogenase, glycolytic enzymes and aminotransferases, which are bound to the cytoplasmic droplets or released during degenerative processes associated with sperm ageing. Most of the proteins that are synthesized and secreted by the epididymis are under the control of steroid hormones [6,7].

Accumulating evidence has been shown that boar epididymis secretes approximately 125 proteins which

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were identified using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis. These secretory proteins of boar epididymis are heterogenic, in terms of both isoelectric point and molecular weight and many of them exhibit adhesive properties [8]. The 2D-PAGE analysis also showed that there was an increase in the concentrations of a few major polypeptides of the plasma membrane of boar spermatozoa during epididymal transit [9]. Moreover, variations in the phosphorylation status of specific proteins have been shown to occur during epididymal transit of the rat spermatozoa [10]. Normally, a high activity of protein kinase is concomitant with increased phosphatase activity and the combined action of these enzymes has been shown to modulate changes in protein phosphorylation. In a recent study, it has been shown that a low activity of protein kinase accompanied by a high activity of serine/threonine phosphatase renders sperm cells immotile [11]. It is noteworthy that boar semen is characterized by a high activity of alkaline and acid phosphatases compared with other species [12]. Four molecular forms of acid phosphatase have been electrophoretically separated in boar seminal plasma; three of them originate in the cauda epididymal fluid and the other one is secreted by the seminal vesicles [13]. Moreover, the molecular form of acid phosphatase, which has been purified from the fluid of boar seminal vesicles shows high affinity for phosphotyrosine residues in protein substrates [14]. However, there is lack of information on the biochemical properties and functions of acid phosphatases originating in the boar epididymis.

The aim of the study was to isolate and characterize a molecular form of epididymal acid phosphatase from boar seminal plasma. This study was focused on the molecular form of acid phosphatase, which displayed the highest electrophoretic mobility. The biochemical properties of the isolated molecular form were also investigated.

2. Materials and methods

2.1. Preparation of seminal plasma

Ejaculates from four sexually mature boars (Polish Large White and Polish Landrace races) were collected using the ''gloved hand'' technique and the gel was removed by filtering through sterile gauze. The seminal plasma obtained after double centrifugations, first at 900 × *g* and then at 10,000 × *g* for 10 min at room temperature, respectively, was used immediately. The seminal plasma was dialyzed overnight at 4 °C against 5 mM TRIS–HCl buffer (pH 8.5) with 0.02% (w/v) sodium azide and then centrifuged at 10,000 × *g* for 15 min at room temperature to separate the precipitate. The resulting supernatant was used for isolation of acid phosphatase.

2.2. Purification procedure of epididymal acid phosphatase from boar seminal plasma

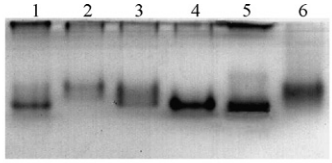
A fast performance liquid chromatography (FPLC) system (Amersham) was used for the chromatography procedures.

2.2.1. Step1: Anion exchange chromatography

Aliquots (100 ml) of the dialyzed seminal vesicle fluid were carefully applied to a DEAE-Sepharose Fast Flow column XK 26/20 (Amersham), equilibrated with 25 mM TRIS–HCl buffer (pH 8.5). The support was eluted with 25 mM TRIS–HCl buffer (pH 8.5) at a flow rate of 10 mL/min. The proteins bound to the chromatography matrix were eluted with linear gradient of 25 mM TRIS–HCl buffer (pH 8.5) containing 0.5 M NaCl. The isolation procedure gave three fractions with acid phosphatase activity and were characterized by the occurrence of different molecular forms. The fraction containing the epididymal molecular form, with the highest electrophoretic mobility (Fig. 1, fraction 3), was used in the next isolation step.

2.2.2. Step 2: Affinity chromatography on Chelating Sepharose Fast Flow-Zn^{2+}

Aliquots (50 ml) of the protein fraction obtained after anion exchange chromatography were loaded on a ''Econo column'' (Bio-Rad Laboratories, USA) packed with 5 ml of Chelating Sepharose Fast Flow (Amersham) gel with immobilized Zn^{2+}-ions. The support was then washed with 25 mM TRIS–HCl, 0.1 M NaCl (pH 8.5). After extensive washing, the bound protein fraction was eluted with 50 mM sodium acetate buffer (pH 4.5). Aliquots of 1 ml protein fractions, corresponding to acid phosphatase activity, were collected



1 – dialyzed seminal plasma, 2 – fraction 1 (DEAE Sepharose), 3 – fraction 2 (DEAE Sepharose), 4 – fraction 3 (DEAE Sepharose), 5 – epididymal fluid, 6 – seminal vesicle fluid

Fig. 1. Polyacrylamide gel electrophoresis (PAGE) of the fractions stained for acid phosphatase activity after DEAE-Sepharose Fast Flow column chromatography of boar seminal plasma.