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Sequestration of bovine seminal plasma proteins by different assemblies of phosphatidylcholine: A new technical approach

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ABSTRACT

Binder of SPerm (BSP) proteins, the main proteins from bovine seminal plasma, are known to partially intercalate into the outer leaflet of the spermatozoa membrane and bind to choline-containing lipids being present therein. This insertion generates a negative effect on semen quality after cryopreservation by inducing an early-stage capacitation of spermatozoa. The assumption of surface properties exhibited by BSP proteins was checked by tensiometry measurements: BSP proteins are highly surface active. This suggests that BSP proteins can reach the interface covered by phospholipids not only by interactions between one and each other but also due to their own surface activity. The insertion of BSP proteins into the lipid domains outer leaflet of spermatozoa was reproduced on a biomimetic system such as Langmuir monolayers. The insertion of BSP proteins can be performed in the compressible fluid domains which contain choline-bearing lipids. Monolayer films were used as well to study the complexation of BSP proteins by two phospholipid assemblies: low density lipoprotein (LDLs) from egg yolk or liposomes produced from egg phospholipids. Irrespective of the phospholipid structure (lipoprotein or liposome), BSP was hindered to alter the structure of the membrane. Only the overall ratio BSP proteins:phosphatidylcholine was important. The difference between the two sequestering agents lies on their surface properties: LDL have a strong tendency to merge with the outer layer whereas liposomes mainly remain in the bulk on the same time scale.

1. Introduction

Binder of sperm proteins (BSP) are a family of proteins known to play roles in the formation of the oviductal sperm storage reservoir and in sperm capacitation, a sperm maturation step which is essential for fertilization [1,2]. In bovine seminal plasma, three majors BSP proteins in 10:1:1 proportion [3] were identified and classified according to the nomenclature of Manjunath et al. [2]: BSP1 [4], BSP3 [5] and BSP5 [6,7], respectively. Their apparent masses are 15–16.5 kDa for BSP1 and BSP3 and 28–30 kDa for BSP5 [6,8]. These three proteins are composed of a unique N-terminal domain followed by two fibronectin type II domains. Only the N-terminal domains of the three BSP proteins differ considerably [4–6]. Each fibronectin type II domain contains a phosphorylcholine binding site [9–14]. Those last ones are essential for the biological roles of BSP proteins. Indeed, the outer leaflet of spermatozoa membrane goes into contact with the seminal plasma which contains BSP proteins during ejaculation [2,15]. BSP proteins bind quickly to the choline-containing phospholipids such as phosphatidylcholine and sphingomyelin present on the sperm membrane [9,12,16–19] and coat the surface of sperm by partially intercalating into the outer leaflet of the sperm plasma membrane [1,13,20]. This is the first crucial step towards the formation of the oviductal sperm storage reservoir and of sperm capacitation later. The fact that BSP proteins are both soluble and able to insert in hydrophobic membranes argues for an amphiphilic character of BSP proteins. Therefore, the first part of this study was designed to test this hypothesis by performing tensiometric measurements.

Early-stage capacitation induced by BSP proteins [21,22] has a negative effect on semen quality after cryopreservation as it results in a loss of cold resistance and freezing tolerance [12,17,23–25].

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Therefore the presence of protectors is essential to maintain sperm quality. Egg-yolk was widely used to sequester BSP proteins [17,26–32]. This sequestration is due to specific and strong interactions between phosphatidylcholine of the outer layer of low-density lipoproteins (LDLs) being present in egg yolk, and BSP proteins [9,12,33,34]. In some cases, the protection of sperm against early-stage capacitation by LDLs was also assumed to result from an exchange between polar lipids of LDLs being rich in phospholipids, and the spermatozoa membrane reduced in lipids and cholesterol due to efflux [29]. Other phospholipids such as soy lecithin are currently used as an alternative to phospholipids from egg yolk. However, in this case, the possible interactions between liposomes and the membranes are not known. Thus, the second part of this study was designed to compare the behaviour of LDLs and liposomes in contact with the lipid membranes. For this purpose a monolayer of lipids at the air–buffer interface was used to mimic the lipid domains of the outer leaflet of the sperm membrane. This method proved to be efficient to reproduce the heterogeneous domains of the lipid domains of the outer leaflet of the bovine spermatozoa membrane [35]. This last one was represented by the main lipid components, namely sphingomyelin, cholesterol, 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (PC) and plasmalogen 1-(1Z-octadecenyl)-2-docosahexaenoyl-sn-glycero-3-phosphocholine (P-PC). In this monolayer, cholesterol and sphingomyelin structure themselves into condensed domains surrounded by a liquid mixture of PC and P-PC at 34 °C. LDLs and liposomes were introduced in the subphase below the monolayer at a pressure close to the relevant to biological membranes [36]. The interactions between the additives and the monolayer were detected by monitoring the changes in surface area. At last, the sequestering properties of LDLs and liposomes towards BSP proteins were compared on the same lipid model of outer leaflet of spermatozoa membranes and in the same conditions.

2. Material and methods

2.1. Materials

Lipids that mainly constitute the outer leaflet of the reconstructed membrane were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and used as received. The compounds 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (PC (16:0/22:6), Mw = 806.1, purity > 99%) and 1-(1Z-octadecenyl)-2-docosahexaenoyl-sn-glycero-3-phosphocholine PC (P-18:0/22:6, Mw = 818.2, purity > 99%) were obtained as chloroform solutions (10 mg mL^{-1}). The stock solutions of PC (16:0/22:6) and P-PC (18:0/22:6) were diluted to obtain 1.25 mg mL^{-1} solutions. Cholesterol from ovine wool (Chol, Mw = 711.0, purity > 98%) and sphingomyelin from chicken egg (SM, Mw = 386.7, purity > 98%) were obtained as powder. Both were solubilized in chloroform (HPLC grade, Carlo Erba, Val de Reuil, France) at a concentration of 1.25 mg mL^{-1}.

The cryoprotective medium consisted of Trizma base (173.0 mM), citric acid mono hydrated (70.0 mM), fructose (56.0 mM), glycerol (6.4%; v/v) and finally antibiotics (penicillin G sodium 391 mg L^{-1}, streptomycin sulphate 856 mg L^{-1}, lincomycin hydrochloride 204 mg L^{-1}, spectinomycin sulfate tetrahydrate 585 mg L^{-1}) (Sigma–Aldrich, St. Quentin Fallavier, France). The pH was adjusted at 6.2 and its osmolarity was approximately 1300.0 mOsm.

2.2. Extraction of LDLs

Low density lipoproteins (LDLs) were extracted from egg yolk according to the protocol of Moussa et al. [31]. After extraction, the concentrated extract of LDLs (22 ± 0.5 g equivalent to 8.36 g of dry LDLs) was diluted in the cryoprotective medium (100 mL). The quantification of proteins in the LDLs sample by the micro BCA protein assay kit (Pierce, Thermoscientific, France) gave 8.9 ± 1.0 mg mL^{-1}. The amount of PC phospholipids in LDLs sample was calculated from the natural proportion of PC in egg (18.4 g of PC/100 g of dry LDLs) given by Anton et al. [33]. It equals to 15.4 g L^{-1} of LDLs sample. LDLs contain also a small fraction of PE (PC/PE 6:1) [33].

2.3. Collect of plasma seminal

Bovine sperm was collected from one Prim Holstein bull and immediately centrifuged twice at 10,000 × *g* to recover the seminal plasma in the supernatant. The amount of proteins of our samples was 88.5 ± 5.0 mg mL^{-1}, as determined by the micro BCA protein assay kit (Pierce, Thermoscientific, France).

2.4. Fabrication of liposomes

Liposomes were produced from an extract of egg phospholipids in the cryoprotective buffer by extrusion using the Mini extruder from Avanti Polar Lipids (Alabaster, Alabama, USA). The extract of egg phospholipids was composed of phosphatidylcholine (73%) and phosphatidylethanolamine (11%) and minor components including triglycerides and sphingomyelin. The extract was hydrated in the cryoprotective buffer overnight at the lipid concentrations of respectively 3.90 mg mL^{-1} (C1), 7.28 mg mL^{-1} (C2), 14.56 mg mL^{-1} (C3) and 21.84 mg mL^{-1} (C4). Then the dispersion was sonicated for 15 min in an ultrasonic bath (Elmasonic S 30H from Elma, Singen, Germany with a power of 275 W). Finally, the dispersion was extruded in an extruder (20 passes through the 100 nm membrane). The distribution size is centred at 120 nm and the Zeta potential value is −6 mV. The amount of liposomes introduced in the presence of BSP was expressed in weight (1.17 mg, 2.18 mg, 4.37 mg and 6.55 mg of lipids for liposome dispersions C1, C2, C3 and C4 respectively). The uncertainty is 0.01 mg.

2.5. Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing conditions. Seminal plasma was dissolved in a pH 6.8-buffer constituted of Tris 0.5 M, SDS 10%, glycerol 30% w/w and bromophenol blue 0.4% to get a 1 mg mL^{-1} solution. Nine percent β-mercaptoethanol v/v was added and samples were vortexed and subsequently heated at 95 °C for 5 min. Then, 10 μL of a low range molecular weight standards (Kit O6U-0511 from Euromedex, Souffelweyersheim, France) and 10 to 25 μL of seminal plasma were loaded on a 15%-polyacrylamide gel (pH 6.8-Tris 0.5 M and SDS 0.10%) coupled with a pH 8.3-migration buffer constituted of Tris 0.025 M, glycine 0.192 M, SDS 0.1%. Electrophoresis was performed at 20 mA and the gels were stained with a Coomassie blue solution G250. The stained gels were finally scanned and the intensity of peaks was evaluated by the Multi Gauge software (version 2.0, Fuji Photo Film Co., Ltd., Japan).

2.6. Surface activity measurements

Surface tension of Gibbs films (formed by diffusion from solutions of seminal plasma to the air–buffer interface) was measured on a 15-well plate (aluminium bottom multiwell plate with Teflon rims) mounted on a Langmuir balance (microtrough XL-LB, Kibron Inc., Helsinki, Finland) equipped with a high sensitive sensor (Wilhelmy method with an alloy wire). The device was placed in a temperature controlled area fixed at 23 °C and was calibrated with