J. Membrane Biol. 211, 185–190 (2006)
DOI: 10.1007/s00232-006-0009-2

The Journal of
Membrane
Biology

Role of Cholesterol, DOTAP, and DPPC in Prostasome/Spermatozoa Interaction and Fusion

Carlo A. Palmerini[1], Cesare Cametti[2], Simona Sennato[2], Diego Gaudino[2], Enrico Carlini[1], Federico Bordi[2], Giuseppe Arienti[1]

[1]Department of Internal Medicine, Laboratory of Biochemistry, University of Perugia, Via del Giochetto, I-06122 Perugia, Italy
[2]Department of Physics, University of Rome "La Sapienza" and National Institute for the Physics of Matter–Unity Rome 1, Piazza A. Moro, 06000, Roma, Italy

Received: 2 February 2006/Revised: 24 July 2006

Abstract. Prostasomes are membranous vesicles present in ejaculated human semen. They are very rich in cholesterol and can interact with spermatozoa. Their physiological roles are still under study. Prostasomes were mixed with liposomes prepared from various lipids, such as *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium (DOTAP), DOTAP/1,2-dipalmytoyl-*sn*-glycero-3-phosphorylcholine (DPPC, 4:1 molar ratio) and DOTAP/cholesterol (4:1, molar ratio) at different pH values (5–8). The mixing of the lipid phases (fusion) was determined by the relief of octadecyl rhodamine B chloride (R_{18}) self-quenching and the radii of the vesicles, by light scattering measurements. The mixing of lipids and the radii of prostasomes were both influenced by the addition of liposome, although in a different manner. The ability of prostasomes (modified by previous treatment with liposomes) to transfer lipid to spermatozoa was also measured. Pretreatment with DOTAP decreased the phenomenon and addition of DPPC abolished it. On the other hand, pretreatment of prostasomes with DOTAP/cholesterol liposomes did not affect the transfer of lipid between prostasome and spermatozoa. Therefore, the ability of vesicles to fuse (or, at least, to exchange the lipid component) was affected by the enrichment in either natural or artificial lipid. This may open new possibilities for the modulation of spermatozoa capacitation and acrosome reaction.

Key words: Cholesterol — DOTAP — DPPC — Fusion — Lipid transfer — Prostasome — Spermatozoa

Correspondence to: Giuseppe Arienti; email: arienti@unipg.it

Introduction

Prostasomes are membranous vesicles present in human semen (Ronquist & Brody, 1985); they are secreted by the prostate gland and contain large amounts of cholesterol and sphingomyelin, whereas phosphatidylcholine is less abundant (Arvidson et al., 1989). Prostasomes are also rich in Ca^{2+}, guanosine diphosphate, adenosine diphosphate and adenosine triphosphate (Fabiani, 1994; Fabiani et al., 1994); and many of their surface proteins possess catalytic activity (Fabiani, 1994) or are involved in the immune response (Fabiani, 1994; Fabiani et al., 1994). The lipid composition and the presence nonlipid components are peculiar and have prompted researchers to investigate the role of these semen particles.

Prostasomes are able to enhance sperm motility (Stegmayr & Ronquist, 1982). They also play a primary role in the liquefaction of semen (Lilja and Laurel 1984) and in immunosuppression (Kelly et al., 1991; Skibinski et al., 1992). Moreover, several studies have addressed the interaction and fusion of prostasomes with spermatozoa (Arienti et al., 1997a, 2001; Palmerini et al., 1999, 2003) and with themselves (Bordi et al., 2001).

Cationic lipid (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium; DOTAP) is used to produce liposomes with a highly positively charged surface, which enhances their interaction with cellular membrane (Campbell et al., 2001). Besides, the interaction with cationic lipids may change the structure and the properties of membranes (Zhao & Feng, 2004). For this reason, DOTAP liposomes have been exploited to deliver various molecules to cells (Senior et al., 1991). Phosphatidylcholine is considered a lipid bilayer stabilizer (Campbell et al., 2001), and cholesterol is important for prostasome function (Arienti et al., 2001).

In this study, we enriched prostasomes with such lipids as DOTAP, DOTAP/cholesterol and DOTAP/1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC), with the aim of evaluating the possible effect of these lipids on the properties of prostasomes and, particularly, their ability to exchange materials with spermatozoa. We found that the liposome-prostasome interactions are rather complex, resulting in either stable or time-decaying structures, according to the charge density of the liposome surface and the pH of the medium. The prostasome-spermatozoa fusion (as measured by vesicle diameter and R_{18} fluorescence self-quenching release) is influenced by the component lipids, indicating the importance of cholesterol in this phenomenon.

Materials and Methods

Materials

Lipids used to prepare liposomes, DOTAP and DPPC, were purchased from Avanti Polar Lipids (Alabaster, AL) and Sigma (St. Louis, MO), respectively. DOTAP (MW 699) and DPPC (MW 734) were used without further purification. Octadecyl rhodamine B chloride (R_{18}) was produced by Molecular Probes (Eugene, OR). HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic] acid), Thesit (dodecylpoly[ethyleneglycolether]$_9$) and MES (2-[*N*-morpholino]-ethanesulfonic acid) were purchased from Boehringer Biochemie (Mannheim, Germany). Sephadex G-50 and Sephadex G-200 were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Other reagents (reagent grade or better) were purchased from common commercial sources.

Preparation of Prostasomes

Fresh human semen was obtained from healthy donors (25–35 years old) and left for 30–40 min at room temperature to liquefy. Semen was then centrifuged at 800 x *g* for 10 min, and the pellet was used to prepare spermatozoa as described below, whereas the supernatant (S_1) was diluted (1:1, by vol) with Tris 30 mmol · liter^{-1} and NaCl 130 mmol · liter^{-1} (adjusted to pH 7.6 with HCl) and centrifuged at 10,000 x *g* for 20 min to eliminate cell debris and residual spermatozoa. The new supernatant (S_2) was then centrifuged at 105,000 x *g* for 90 min. The supernatant (S_3) was discarded and the pellet (P_3), containing prostasomes and amorphous material, was suspended in the same buffer to have about 1.0–1.5 mg protein · ml^{-1}. Prostasomes were purified from amorphous material by chromatography on a Sephadex G-200 column (1.5 x 30 cm) preequilibrated with Tris 30 mmol · liter^{-1} + NaCl 130 mmol · liter^{-1} (adjusted to pH 7.6 with HCl) (Arienti et al., 2002; Crommelin & Schreirer 1994).

Preparation of Spermatozoa

Fresh human semen was obtained from healthy donors and treated as described above. Spermatozoa were collected by the swim-up procedure described by Lopata et al. (1976), partially modified. Briefly, Earle's balanced salt solution (1 ml) also containing 1% (w/v) bovine serum albumin was layered over 1 ml of seminal fluid. Samples were incubated for 60 min at 37°C in a thermostat-regulated CO_2 chamber. The upper layer, rich in motile spermatozoa, was collected.

Preparation of Liposomes

DOTAP liposomes, DOTAP/cholesterol mixed liposomes (molar ratio 4:1) and DOTAP/DPPC mixed liposomes (molar ratio 4:1) were obtained by dissolving 37.2 μmoles of DOTAP (+ cholesterol or + DPPC, when necessary) in 10 ml methanol:chloroform (1:1 v/v). Solutions were then placed in a glass vessel and allowed to form a dry film after evaporation of the solvent overnight and under vacuum with a rotating apparatus. The film was then hydrated with 15 ml of water at 44°C for 1 h. The resulting suspension was sonicated (pulsed-power mode) for 1 h at 25°C (Vibra-Cell; Sonics Sonicating Apparatus, New Town, CT), until the preparation appeared to be optically transparent in white light. The liposomal suspensions were filtered through a 0.4 μm polycarbonate filter (Millipore, Bedford, MA). This procedure produced a homogeneous unilamellar liposomal suspension (Crommelin & Schreirer 1994).

Dynamic Light Scattering Measurements

Size and size distribution of prostasomes and liposome-prostasome aggregates were determined by dynamic light scattering measurements carried out with a Brookhaven BI9000AT logarithmic correlator (Brookhaven Instr. Corp., Long Island, NY) at 25°C.

In dynamic light scattering experiments, the Laplace inversion of the first-order electric field time autocorrelation function yields the translational diffusion coefficients, which give the hydrodynamic radius R_H of the diffusing aggregates by the Stokes-Einstein relationship. Details of the method are reported elsewhere (Schmitz, 1990).

Assay of Lipid Mixing Between Particles

The insertion of R_{18} into prostasomes was performed as described (Hoekstra et al., 1984). The probe was dissolved in ethanol (1 mg · ml^{-1}), and 50 μl of this solution were added to 1 ml of vesicle suspension. The mixture was then kept in the dark for 1 h at room temperature. To eliminate noninserted R_{18}, prostasomes were chromatographed on a Sephadex G-25 column (0.5 x 25 cm) and eluted with 130 mmol · liter^{-1} NaCl and 30 mmol · liter^{-1} Tris-HCl (pH 7.4). The fluorescence of these preparations was stable for many hours.

The mixing of lipids was tested by the relief of R_{18} fluorescence self-quenching, which monitors lipid mixing (Arrastua et al., 2003; Arienti et al., 1997a). The assay was performed in a cuvette containing 320 mmol · liter^{-1} sucrose, 20 mmol · liter^{-1} MES or 2 mmol · liter^{-1} HEPES at the required pH values (final volume 1.85 ml). Labeled prostasomes (50 μl) were then added. The process was started with unlabeled liposomes (100 μl) and monitored following the increments of fluorescence at 580 nm (excitation 560 nm).

Spermatozoa were tested against labeled prostasomes (either native or pretreated with liposomes), as described above.

The calibration of the assay was performed taking as 100% the fluorescence produced after the addition of 1% (final concentration) Thesit; fluorescence did not increase upon further additions of the detergent. No increase in fluorescence was detected before the addition of unlabeled vesicles.

The extent of fusion (taking as 100% the complete intermixing of lipid phases) was calculated from the percentage of fluorescence self-quenching relief using the following relationship (Corazzi et al., 1991): $Fu = Fl(1 + r/u)$, were Fu is the percentage of fusion, Fl is the percentage of fluorescence quenching relief, r is the amount of labeled lipid (as mole) and u is the amount of unlabeled lipid (as mole).

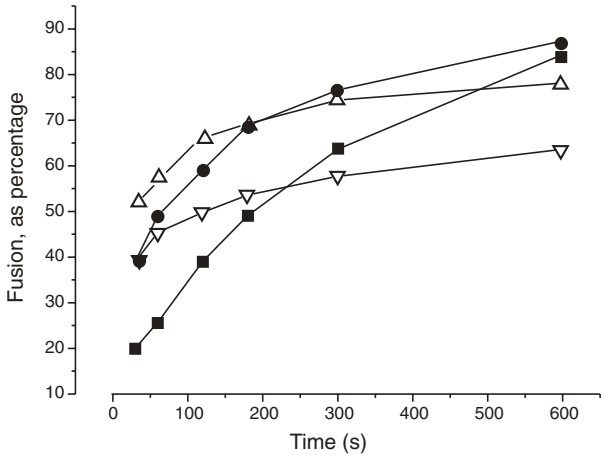

Fig. 1. Interaction of DOTAP liposomes and prostasomes. DOTAP liposomes were mixed with prostasomes (2:1 molar ratio) and incubated for the indicated times at 25°C at various pH values. Prostasomes were previously labeled with R_{18}, and the percentage of fusion was calculated from the relief of R_{18} fluorescence self-quenching. ■, pH 5.0; ●, pH 6.0; Δ, pH 7.0; ∇, pH 8.0. Data are averages of three experiments. Standard deviations did not exceed 10% and were omitted for clarity.

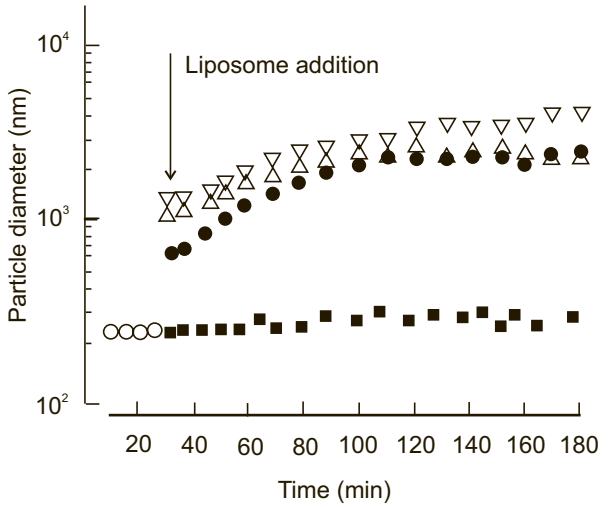

Fig. 2. Time-related modifications of the hydrodynamic radii of liposome/prostasome aggregates at different pH values. Mixed liposomes (DOTAP/DPCC molar ratio 4:1) were added to the prostasome suspension at the time marked by the *vertical arrow*. ■, pH 5.0; ●, pH 6.0; Δ, pH 7.0; ∇, pH 8.0; ○, before addition of liposomes. Data are averages of three experiments. Standard deviations did not exceed 10% and were omitted for clarity.

Analyses

Phospholipid and cholesterol were determined as described (Bartlett, 1959; Rudel & Morris, 1973).

Results

DOTAP Liposome-Prostasome Interaction

We investigated by dynamic light scattering measurements the aggregates formed by prostasomes in the presence of DOTAP liposomes (DOTAP/prostasome lipid phosphorus, molar ratio 2:1) at different pH values.

The hydrodynamic radii of the particles resulting from the interaction between liposomes and prostasomes did not appreciably change (*results not shown*), but the relief of R_{18} fluorescence self-quenching indicated extensive mixing of lipid between the vesicles (DOTAP liposomes and R_{18}-labeled prostasomes) at each examined pH value (5–8) (Fig. 1). The percentage of lipid mixing did not depend on pH values. Therefore, the lipid transfer between liposomes and prostasomes that occurred in these conditions appears to be unrelated to the mechanism of fusion of prostasomes to themselves (Bordi et al., 2001) and spermatozoa, previously described in our laboratories (Arienti et al., 1997a).

Mixed DOTAP/DPPC Liposome-Prostasome Interactions

A different picture emerges from the interaction of prostasome and mixed DOTAP/DPPC (molar ratio 4:1) liposomes. In this case, owing to the zwitterionic character of DPPC, the surface charge density is reduced, thus favoring the formation of aggregates of larger size (up to 1 μm), as measured by light scattering. The results are summarized in Figure 2, showing that, for all the investigated pH values with the exception of 5.0, the size of prostasomes increased, producing aggregates that reached a steady-state condition in about 120 min.

In a further set of experiments, R_{18}-labeled prostasomes were mixed with DOTAP/DPPC liposomes (Fig. 3). The lipid phase intermixing depended on the pH values of the mixtures, the largest extent being obtained at pH 5.0 and the lowest at pH 8.0. Our data indicate that the phenomenon was noticeably lower and its time course was slower when compared to the results obtained with DOTAP liposomes.

Mixed DOTAP/Cholesterol (Molar Ratio 4:1) Liposome-Prostasome Interactions

Cholesterol is abundant in prostasomes and plays an important role in the activation of spermatozoa (Arienti et al., 2001). This prompted us to investigate the effect of cholesterol addition to DOTAP liposomes on vesicle properties. The results did not show any variation of the hydrodynamic radii of the resulting particles after liposome-prostasome interaction. Yet, the mixing of the lipid phases of vesicles (R_{18} method, Fig. 4) became much lower and slower following the addition of cholesterol to DOTAP

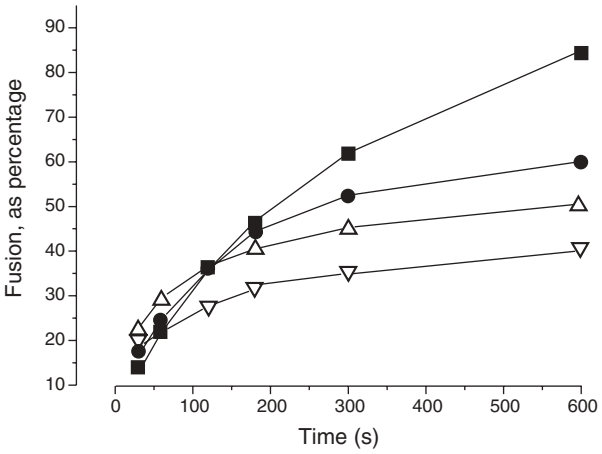

Fig. 3. Interaction between DOTAP/DPPC liposomes and prostasomes. Liposomes (DOTAP/DPPC molar ratio 4:1) were mixed with prostasomes (2:1 molar ratio) and incubated for the indicated times at 25°C at various pH values. Prostasomes were previously labeled with R_{18}, and the percentage of fusion was calculated from the relief of R_{18} fluorescence self-quenching. ■, pH 5.0; ●, pH 6.0; Δ, pH 7.0; ∇, pH 8.0. Data are averages of three experiments. Standard deviations did not exceed 10% and were omitted for clarity.

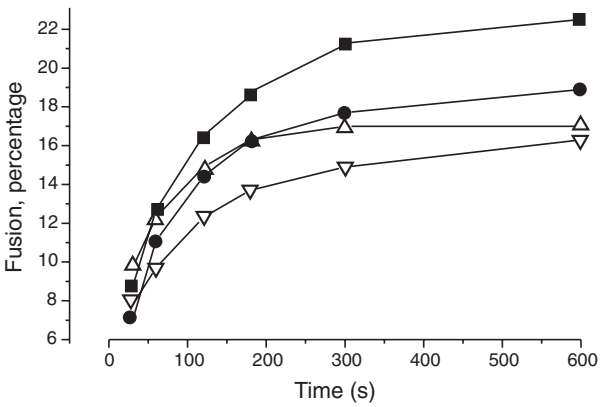

Fig. 4. Interaction between DOTAP/cholesterol liposomes and prostasomes. Liposomes (DOTAP/cholesterol molar ratio 4:1) were mixed with prostasomes (2:1 molar ratio) and incubated for the indicated times at 25°C at various pH values. Prostasomes were previously labeled with R_{18}, and the percentage of fusion was calculated from the relief of R_{18} fluorescence self-quenching. ■, pH 5.0; ●, pH 6.0; Δ, pH 7.0; ∇, pH 8.0. Data are averages of three experiments. Standard deviations did not exceed 10% and were omitted for clarity.

liposomes. The influence of pH values on this phenomenon was rather small.

Lipid Exchange Between Modified Prostasomes and Spermatozoa

With the above-described experiments we could modify the lipid composition and the size of prostasomes. We then investigated the ability of the prostasomes so modified to fuse to spermatozoa (Figs. 5 and 6).

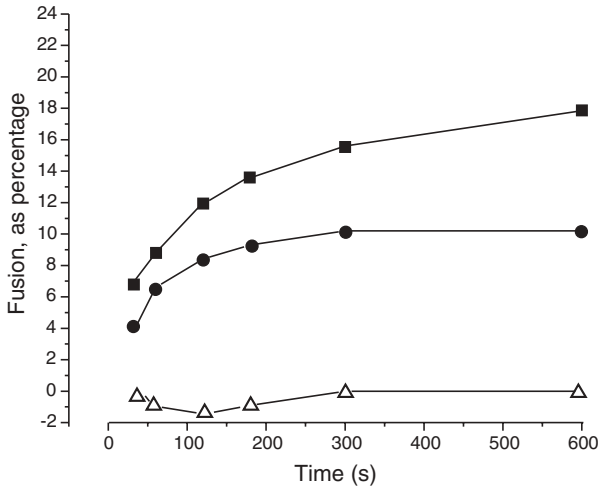

Fig. 5. Fusion of DOTAP- or DOTAP/DPPC-enriched prostasomes and spermatozoa. Prostasomes were mixed with spermatozoa (2:1 lipid molar ratio) and incubated for the indicated times at 25°C at pH 5.0. Prostasomes were previously enriched in DOTAP or in DOTAP/DPPC by fusion with DOTAP or DOTAP/DPPC prostasomes. They were then labeled with R_{18}, and the percentage of fusion was calculated from the relief of R_{18} fluorescence self-quenching. See text for details. ■, Fusion of spermatozoa to non-modified prostasomes; ●, fusion of spermatozoa to prostasomes enriched in DOTAP; Δ, fusion of spermatozoa to prostasomes enriched in DOTAP/DPPC. Data are averages of three experiments. Standard deviations did not exceed 10% and were omitted for clarity.

The transfer of lipid between spermatozoa and prostasomes (enriched or not with DOTAP or DOTAP/DPPC liposomes) was absent at pH 8.0, confirming previously reported data (Arienti et al., 1997a; *data not shown*). At pH 5.0, the lipid transfer between prostasomes and spermatozoa was present but the modification of prostasomes by preexposure to DOTAP liposomes decreased the extent of this phenomenon. In the case of DOTAP/DPPC (molar ratio 4:1), the prostasome fusion with spermatozoa was practically abolished (Fig. 5).

The addition of cholesterol to DOTAP liposomes made treated prostasomes similar to native prostasomes, with respect to their ability to interact with spermatozoa (Fig. 6). This phenomenon underlines the importance of cholesterol in prostasome-spermatozoa interactions.

Discussion

The measurement of hydrodynamic radii (Bordi et al., 2001) of the particles and the exchange of lipid material between vesicles have been proposed as

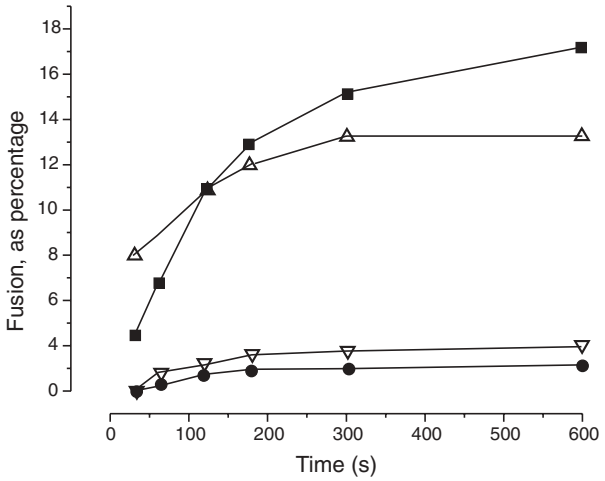

Fig. 6. Fusion of DOTAP/cholesterol-enriched prostasomes and spermatozoa. Prostasomes were enriched with DOTAP and cholesterol through incubation with DOTAP/cholesterol prostasomes as described in Materials and Methods. They were then mixed with spermatozoa (2:1 lipid molar ratio) and incubated for the indicated times at 25°C. See text for details. Prostasomes were previously labeled with R_{18}, and the percentage of fusion was calculated from the relief of R_{18} fluorescence self-quenching. ■, Spermatozoa were fused with untreated prostasomes; ●, pH 8.0, spermatozoa were fused with untreated prostasomes; Δ, pH 5.0, spermatozoa were fused with DOTAP/cholesterol-enriched prostasomes; ∇, pH 8.0, spermatozoa were fused with DOTAP/cholesterol-enriched prostasomes. Data are averages of three experiments. Standard deviations did not exceed 10% and were omitted for clarity.

methods to investigate membrane fusion, although the increase in diameter can also be related to the aggregation of particles. Similarly, R_{18} is a lipophilic dye, whose fluorescence self-quenching releases upon dilution (Hoekstra et al., 1984). Therefore, if the fluorescence self-quenching decreases upon the mixing of two particle populations, only one of which is loaded with R_{18}, it may be assumed that a transfer of lipid is present. Many other methods have been proposed to detect membrane fusion, and the meaning of this word is often related to the method used to measure it (Duzgunes & Wilschut, 1993; Hoekstra & Duzgunes, 1993). The Oterbium-dipicolinate method is probably more selective, measuring the mixing of the water phases inside the particles; but the particular type of preparation used in this report did not allow use of this method (Corazzi et al., 1989).

The enrichment of prostasomes with DOTAP liposomes (sometimes containing phosphatidylcholine or cholesterol as well) was obtained by mixing prostasomes and liposomes in various experimental conditions. In most instances, we found a transfer of lipid, as measured by the relief of R_{18} fluorescence self-quenching, whereas an increase of particle size was noticed only with DOTAP/DPPC liposomes. Therefore, the size of resulting particles and the transfer of the dye appear to be unrelated phenomena. The increase of particle size may be due to aggregation, whereas the R_{18} method indicates that the particles exchange lipid; the different nature of the methods employed here may offer an easy explanation of the experimental observations.

The different properties of the lipids used in this work may explain the behavior of the liposome-prostasome interaction. DOTAP is a cationic detergent whose presence stimulates the transfer of lipid between vesicles, independent of pH values. On the other hand, the addition of DPPC (a zwitterionic compound) to DOTAP changes the properties of the system. The size of the particles is influenced and the transfer of R_{18} between prostasomes and liposomes becomes pH-dependent (highest at the lowest pH values). The effect may be due to the dissociation constants of ionizable groups (Duzgunes & Nir, 1999).

We also examined DPPC/cholesterol liposomes. The presence of cholesterol produces small changes of the properties of liposomes made of DOTAP alone, except for the fact that the addition of cholesterol decreases dramatically the transfer of the dye between the particles. The lipophilic nature of cholesterol and the lack of ionizable groups in this molecule may explain the effect.

We investigated the fusion of spermatozoa and prostasomes (Arienti et al., 1997a) after the enrichment of these vesicles with various lipids: DOTAP, DOTAP/DPPC (molar ratio 4:1) and DOTAP/cholesterol (molar ratio 4:1). An interesting aspect of these experiments is the modification of treated prostasomes to exchange lipid with spermatozoa. Indeed, pretreatment with DOTAP/DPPC liposomes abolished the fusion of prostasomes and spermatozoa (at pH 5.0). On the other hand, if prostasomes are pretreated with DOTAP/cholesterol liposomes, they retain their ability to fuse to spermatozoa. These effects may be explained on the grounds that cholesterol may "dilute" the charge of cationic detergents; on the other hand, the transfer of lipids to prostasomes is much higher if cholesterol is omitted from liposomes. Therefore, prostasomes are less disturbed if they are tested after treatment with cholesterol-containing liposomes.

The fusion between prostasomes and spermatozoa is a complex process depending on both the protein (Arienti et al., 1997b, 1997c, 1997d) and lipid composition of prostasomes. It may be interesting to observe that native prostasomes are very rich in cholesterol and that this lipid has important effects on spermatozoa because it influences capacitation and acrosomal reaction (Duzgunes, 1988).

The possibility of changing the properties of prostasomes by mixing them with artificial and natural lipids may prove of some interest since the lipid composition of spermatozoa is influenced by the presence of prostasomes in the medium and, in turn,

influences the activation of these cells, which is necessary for the fecundation of the ovum.

References

Arienti, G., Carlini, E., Palmerini, C.A. 1997a. Fusion of human sperm to prostasomes at acidic pH. *J. Membr. Biol.* **155:**89–94

Arienti, G., Carlini, E., Saccardi, C., et al. 2002. Nitric oxide and fusion with prostasomes increase cytosolic calcium in progesterone-stimulated sperm. *Arch. Biochem. Biophys.* **402:**255–258

Arienti, G., Carlini, E., Verdacchi, R., et al. 1997b. Prostasome to sperm transfer of CD13/aminopeptidase N (EC 3.4.11.2). *Biochim. Biophys. Acta.* **1336:**533–538

Arienti, G., Carlini, E., Verdacchi, R., et al. 1997c. Transfer of aminopeptidase activity from prostasomes to sperm. *Biochim. Biophys. Acta.* **1336:**269–274

Arienti, G., Nicolucci, A., Santi, F., et al. 2001. Progesterone-induced increase of sperm cytosolic calcium is enhanced by previous fusion of spermatozoa to prostasomes. *Cell Calcium* **30:**222–227

Arienti, G., Polci, A., Carlini, E., et al. 1997d. Transfer of CD26/dipeptidyl peptidase IV (E.C. 3.5.4.4) from prostasomes to sperm. *FEBS Lett.* **410:**343–346

Arrastua, L., San, S.E., Quincoces, A.F., et al. 2003. In vitro fusion between Saccharomyces cerevisiae secretory vesicles and cytoplasmic-side-out plasma membrane vesicles. *Biochem. J.* **370:**641–649

Arvidson, G., Ronquist, G., Wikander, G., et al. 1989. Human prostasome membranes exhibit very high cholesterol/phospholipid ratios yielding high molecular ordering. *Biochim. Biophys. Acta.* **984:**167–173

Bartlett, G.R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:**466–468

Bordi, F., Cametti, C., De Luca, F., et al. 2001. Hydrodynamic radii and lipid transfer in prostasome self-fusion. *Arch. Biochem. Biophys.* **396:**10–15

Campbell, R.B., Balasubramanian, S.V., Straubinger, R. 2001. Phospholipid-cationic lipid interactions: influences on membrane and vesicle properties. *Biochim. Biophys. Acta.* **1512:**27–39

Corazzi, L., Fratto, G., Pistolesi, R., et al. 1989. Fusion of liposomes and rat brain microsomes examined by two assays. *J. Membr. Biol.* **112:**123–129

Corazzi, L., Pistolesi, R., Arienti, G. 1991. The fusion of liposomes to rat brain microsomal membranes regulates phosphatidylserine synthesis. *J. Neurochem.* **56:**207–212

Crommelin, D.J.A., Schreirer, H. 1994. Liposomes. *In:* Kreuter, J., editor. Colloidal Drug Delivery Systems. pp 73–190, Maecell Dekker, New York

Duzgunes, N., Nir, S. 1999. Mechanisms and kinetics of liposome-cell interactions. *Adv. Drug Deliv. Rev.* **40:**3–18

Duzgunes, N. 1988. Cholesterol and membrane fusion. *In:* Yeagle, P.L., editor. Biology of cholesterl. pp 197–212, CRC Press, Boca Raton

Duzgunes, N., Wilschut, J. 1993. Fusion assays monitoring intermixing of aqueous contents. *Methods Enzymol.* **220:**3–14

Fabiani, R. 1994. Functional and biochemical characteristics of human prostasomes. Minireview based on a doctoral thesis. Ups. *J. Med. Sci.* **99:**73–111

Fabiani, R., Johansson, L., Lundkvist, O., et al. 1994. Promotive effect by prostasomes on normal human spermatozoa exhibiting no forward motility due to buffer washings. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **57:**181–188

Hoekstra, D., de Boer, T., Klappe, K., et al. 1984. Fluorescent method for measuring the kinetic of fusion between biological membranes. *Biochemistry* **23:**5675–5681

Hoekstra, D., Duzgunes, N. 1993. Lipid mixing assays to determine fusion in liposome systems. *Methods Enzymol.* **220:**15–32

Kelly, R.W., Holland, P., Skibinski, G., et al. 1991. Extracellular organelles (prostasomes) are immunosuppressive components of human semen. *Clin. Exp. Immunol.* **86:**550–556

Lilja, H., Laurel, C.B. 1984. Liquefaction of coagulated human semen. *Scand. J. Clin. Lab. Invest.* **44:**447–452

Lopata, A., Patullo, M.G., Chang, A., et al. 1976. A method for collecting motile spermatozoa from human semen. *Fertil. Steril.* **27:**265–275

Palmerini, C.A., Carlini, E., Nicolucci, A., et al. 1999. Increase of human spermatozoa intracellular Ca^{2+} concentration after fusion with prostasomes. *Cell Calcium* **25:**291–296

Palmerini, C.A., Saccardi, C., Carlini, E., et al. 2003. Fusion of prostasomes to human spermatozoa stimulates the acrosome reaction. *Fertil. Steril.* **80:**1–4

Ronquist, G., Brody, I. 1985. The prostasome: its secretion and function in man. *Biochim. Biophys. Acta.* **822:**203–218

Rudel, L.L., Morris, M.D. 1973. Determination of cholesterol using *o*-phtalaldehyde. *J. Lipid Res.* **14:**364–366

Schmitz, K.S. 1990. Introduction to quasi elastic light scattering by macromolecules. Academic Press, New York

Senior, J.H., Trimble, K.R., Maskiewicz, R. 1991. Interaction of positively-charged liposomes with blood: implications for their application in vivo. *Biochim. Biophys. Acta.* **1070:**173–179

Skibinski, G., Kelly, R.W., Harkiss, D., et al. 1992. Immunosuppression by human seminal plasma–extracellular organelles (prostasomes) modulate activity of phagocytic cells. *Am. J. Reprod. Immunol.* **28:**97–103

Stegmayr, B., Ronquist, G. 1982. Promotive effect on human sperm progressive motility by prostasomes. *Urol. Res.* **10:**253–257

Zhao, L., Feng, S.S. 2004. Effects of lipid chain length on molecular interactions between paclitaxel and phospholipid within model biomembranes. *J. Colloid. Interface Sci.* **274:**55–68