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BULL SPERM PLASMA AND ACROSOMAL MEMBRANES: FLUORESCENCE STUDIES OF LIPID PHASE FLUIDITY

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SUMMARY Bull sperm plasma and outer acrosomal membranes have been isolated by discontinuous sucrose density gradient centrifugation and Ca^{2+}-ATPase activity has been determined for both the membranes. Pyrene excimer fluorescence and diphenylhexatriene fluorescence polarization studies show that the lipid phase of the sperm plasma membranes is more fluid than the lipids of the outer acrosomal membranes. Approximately, a three fold increase in the cholesterol content has been found in the outer acrosomal membranes as compared to that in the plasma membranes.

INTRODUCTION

The plasma membrane of spermatozoa maintains the integrity of the cell and undergoes many prominent alterations during epididymal maturation (1). The acrosomal membranes form the boundary of acrosomal vesicles, which contains the hydrolytic enzymes necessary for the penetration of the ovum. The selective fusion and vesiculation of the outer acrosomal and plasma membranes is part of the acrosome reaction, an obligatory event prior to fertilization (2). Plasma membrane components like acetylcholinesterase and Na^+, K^+-ATPase have also been implicated in modulation of sperm motility (3). Studies on sperm plasma and acrosomal membranes would therefore be valuable in understanding the phenomena of maturation,

Abbreviations : HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid ; DPH, 1,6-Diphenyl-1,3-5-hexatriene.

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motility and the acrosome reaction in spermatozoa. In this report we describe mainly, a comparison of the lipid phase fluidities of plasma and outer acrosomal membranes of bull sperm. The plasma membrane is shown to have a more fluid lipid phase than the outer acrosomal membranes, using pyrene excimer fluorescence and DPH fluorescence polarization as probes. A correlation between membrane cholesterol content and fluidity is noted.

MATERIALS AND METHODS

Isolation of membranes and assays

Bull semen was diluted with two volumes of 0.15 M NaCl-5mM HEPES, pH 7.0. Sperm cells were isolated from diluted semen and membranes were prepared, essentially as described by Zahler and Doak (4). In a typical preparation, 30 ml. of bull semen yielded 18-20 mg. of plasma membrane protein and $\sim$ 8 mg. of acrosomal membrane protein. Protein (5), inorganic phosphate (6) concentrations and Ca^{2+}-ATPase activity (7), were determined by standard procedures. The method of Bligh and Dyer (8) was used for total lipid extraction. Cholesterol was estimated as in (9).

Fluorescence studies

Fluorescence spectra were recorded on a Perkin-Elmer MPF-44A fluorescence spectrophotometer, operated in the ratio mode, with 5 nm, excitation and emission bandpass. Pyrene was incorporated into membranes by rapid mixing of a stock solution in ethanol (2 mM) with the membrane suspension, such that the alcohol concentration did not exceed 1% . The excitation wave length used was 333 nm. For DPH polarization experiments, a stock solution of 4 mM DPH in tetrahydrofuran was diluted 2000 fold into membranes, while mixing thoroughly and then left at 37°C for 30 minutes. Measurements were made using λ (excitation) = 355 nm and λ (emission) = 430 nm. All solutions for fluorescence studies were prepared in 10 mM Tris-HCl, 7.4 buffer. A protein concentration of 100 μg/ml was used.

RESULTS AND DISCUSSION

The subcellular fractionation of sperm yielded two membrane fractions, of which, one banded above 1.3 M sucrose and the other at the 1.3 M/1.75 M sucrose interface. These have been characterised previously, as plasma and outer acrosomal membranes, respectively, by enzymatic and microscopic analyses (4,10,11). We have earlier identified

Table 1

Ca^{2+}-ATPase activity, DPH polarization and cholesterol content of plasma and acrosomal membranes[a] of bull sperm.

Membrane	Ca^{2+}-ATPase[b]	Polarization 'p'	Cholesterol[c]
SPM	47.0	0.15 (±0.01)	18 (± 3.5)
SAM	0	0.23 (±0.01)	61 (±12.7)

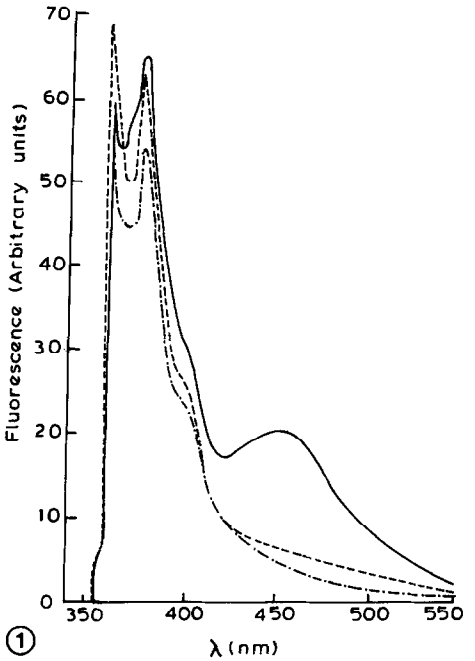
[a] Results shown are the average of experiments with a minimum of three different membrane preparations.

[b] Activity expressed in μmoles of Pi liberated/mg protein/hr.

[c] Expressed in μg/mg protein.

the presence of a Ca^{2+}-ATPase, exclusively associated with the plasma membrane fraction of bull sperm and suggested that the enzyme could serve as marker for the cell surface membranes (11). The enzyme activity in the isolated plasma and outer acrosomal membranes is summarized in Table 1. Using the Ca^{2+}-ATPase levels, the recovery of plasma membranes by this procedure, is estimated to be about 25% . Contamination by mitochondria and cytoplasmic droplets was insignificant as judged by the low levels of succinate dehydrogenase and α-mannosidase in the membrane fractions.

Cholesterol is known to influence the structure and function of biological membranes (12). For example, alteration of the cholesterol level in erythrocyte membranes leads to changes in ionic permeability (13,14), microviscosity (15,16), lateral diffusion (17) and protein-lipid interaction (15). It is seen from Table 1, that the membrane cholesterol content of outer acrosomal membranes is significantly higher than that of the plasma membranes. This difference may have considerable '*in vivo*' significance,



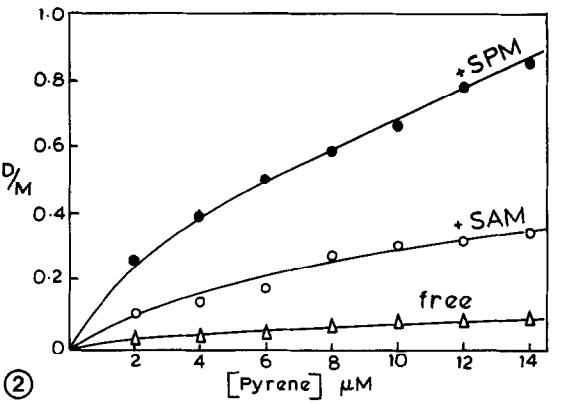


Figure 1. Uncorrected emission spectra of pyrene (4 μM) in membranes. Protein concentration : 100 μg/ml. All solutions in 10 mM Tris-HCl, pH 7.4.
- SPM ; - - - SAM ; -.-. free pyrene.

Figure 2. Dimer/monomer (D/M) intensity ratio for pyrene incorporated into membranes, as a function of pyrene concentration. Protein concentration : 100 μg/ml. All solutions in 10 mM Tris-HCl, pH 7.4.

since, membrane-associated cholesterol is known to have a profound influence in fusion phenomena (18).

Pyrene excimer fluorescence

Lipid phase fluidity can be conveniently measured by following the pyrene excimer fluorescence emission intensity at 470 nm; the formation of excimers being related to the ease of lateral diffusion of pyrene in the lipid layer (19,20). Fig.1 shows the emission spectra of pyrene, dispersed in aqueous buffer and in the SPM and SAM fractions. It can be seen that the excimer band is very weak in the SAM fraction. Fig.2 shows the dependence of the Dimer/Monomer (D/M) ratio on pyrene concentration, for the two membrane systems. Again,

it is clear that, over the concentration range studied, excimer formation is more facile in the SPM fraction, as compared to the SAM fraction. These results suggest that SPM has a more fluid lipid phase than SAM.

The fluorescence polarization value (p) for DPH reflects directly, the rotational freedom of the DPH molecule in the lipid phase (21). The polarization values for DPH in sperm plasma and acrosomal membranes are summarized in Table 1. The significantly higher p value for the acrosomal fraction suggests that the lipid phase in this membrane is more ordered than in the plasma membranes.

Liposome-mediated modifications in the cholesterol content of erythrocyte membranes have been monitored using DPH and a direct correlation between the polarization values and lipid phase fluidity has been established (22). It is noted that the 'p' values of sperm plasma and acrosomal membranes are in good agreement with the observed lower cholesterol content of plasma membranes as compared to the acrosomal fraction.

The apparent fluidity of sperm plasma membranes is of significance in view of its important role in epididymal maturation, capacitation and acrosome reaction (1). The outer acrosomal membranes are responsible for sequestering the hydrolytic enzymes of the acrosome, until the occurrence of the "acrosome reaction". It is of interest therefore to note that this membrane is considerably more rigid in its lipid phase, presumably because of its primary function as a barrier. The results presented above provide a clear evidence for marked differences in the lipid phase mobilities, in the plasma and acrosomal membranes of spermatozoa.

These membranes fuse and vesiculate during the acrosome reaction (2), a process accompanied by an influx of Ca^{2+} into the cell. The role of Ca^{2+} in modulating membrane fluidity, inducing phase separation in the lipid bilayer and facilitating membrane fusion has been stressed (23). Addition of Ca^{2+} upto 10 mM did not have any effect on the pyrene excimer fluorescence of isolated sperm plasma and acrosomal membranes. It remains to be established whether the differential fluidity of the plasma and acrosomal membranes and the possible effect of calcium on their structure, has any direct relevance to the molecular mechanism of the acrosome reaction.

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