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Phospholipase-C sensitive GPI-anchored proteins of goat sperm: possible role in sperm protection

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Abstract

The role of glycosylphosphatidylinositol (GPI)-anchored sperm proteins in reproduction has been investigated. SDS-polyacrylamide gels (PAGE) analysis of goat sperm (*Capra indica*) indicated that several GPI-anchored proteins were released by phosphatidylinositol-specific phospholipase-C (PI-PLC) treatment. The distribution of this category of PI-PLC-sensitive GPI-anchored proteins on the surface of sperm was examined by indirect immunofluorescence. The fluorescence microscopic study clearly demonstrated that the PI-PLC-sensitive GPI-anchored proteins are confined predominantly to the head region of goat sperm. Further experiments were conducted on intact and PI-PLC treated sperm in order to decipher the function of GPI proteins. Co-incubation of sperm with peritoneal macrophages led to the enhanced phagocytosis of PI-PLC treated sperm by macrophages compared with the untreated intact sperm. Transmission electron micrographs of the macrophages acquired from the phagocytosis assay are provided to corroborate the same. From the results obtained it is inferred that one or more of the PI-PLC-sensitive GPI-anchored proteins on the sperm surface could act as protection factor(s) that shield the sperm from macrophages.

Keywords: Sperm; GPI protein; PI-PLC; SDS-PAGE; Immunofluorescence; Transmission electron microscopy (TEM); Phagocytosis; Macrophages

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1. Introduction

It is now widely accepted that proteins of sperm plasma membrane are functional molecules arranged in varied fashion; some embedded (integral) and some others loosely held (peripheral). The glycosylphosphatidylinositol (GPI)-anchored class of integral protein is tethered on the outer leaflet of the membrane lipid bilayer. The GPI structure consists of a glycan-bridge (Manα1–2 Manα1–6 Manα1–4GlcN–NH_2) between phosphatidylinositol and phosphoethanolamine. The phosphatidyl inositol is embedded in the membrane and the glycan-bridge along with the phosphoethanolamine, which is in amide linkage to the C-terminus of the protein, is displayed on the non-cytosolic side of the membrane (Mayor et al., 1990a,b; Hooper, 2001; Ikezawa, 2002).

The attachment of proteins to membranes through the GPI anchor provides several structural advantages. First, its existence in the outer leaflet of the bilayer allows lateral mobility of the molecules that is not restricted by cytoskeletal elements (Ikezawa, 2002). Second, the proteins can be released from their anchors by the action of specific enzymes, the phospholipases-C and -D (Low, 1992). Third, the anchor makes it possible to link a range of proteins as varied as enzymes, receptors, adhesion molecules, differentiation antigens and other biologically active proteins to the surface of membranes (Pereira et al., 2003). Finally, the protein along with the anchor could be transferred from one cell to another through vesicles (Arienti et al., 1997a,b) and phospholipid transfer proteins (Wirtz, 1997). Thus, GPI anchors can facilitate cellular functions like intracellular sorting of proteins (Nosjean et al., 1997; Muniz and Riezman, 2000), transmembrane signaling (Horejsi et al., 1999) and the process of potocytosis (Anderson, 1998; Grunfelder et al., 2003).

The structural analysis of GPI-anchored proteins suggests that the protein component is highly variable while the rest of the molecule is conserved. It is imperative for the sperm to economize on the number of protein molecules on its surface primarily because of its miniscule size and absence of the machinery for protein synthesis. Since GPI acts as the anchor for a variety of proteins, it provides an opportunity for selectively deleting existing proteins and acquiring new ones during its journey to meet with the egg making this class of proteins especially interesting (Pereira et al., 2003).

Over the past few years, a number of functions have been attributed to the GPI-anchored proteins present on sperm membranes. The role of GPI proteins as ectoenzymes has received the most attention. Among them are the hyaluronidase (Thaler and Cardullo, 1995), 5′nucleotidase (Schiemann et al., 1994) and alpha-mannosidase (Kuno et al., 2000). These molecules are believed not only to possess catalytic activity but also interact with molecules on the egg in a receptor–ligand fashion to initiate signaling (Cherr et al., 2001). Sperm GPI proteins with exclusive receptor function has also been reported (Shetty et al., 2003). In one instance, a sperm GPI-anchor has been shown to act as carrier for a ‘decapacitation factor’ (DF) that is released from the sperm surface during the process of capacitation (Fraser, 1998). A large number of molecules, particularly the cluster of differentiation (CD) class of GPI proteins associated with the lymphocytes, are also found to exist on the surface of sperm. They are believed to protect the sperm against complement attack (Kirchhoff and Hale, 1996). More recently, a GPI protein (MAK248) has been reported to possess protease inhibitory activity (Yudin et al., 2002).