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Mouse Epididymal Spam1 (PH-20) Is Released in the Luminal Fluid With its Lipid Anchor

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Previously we demonstrated that the murine sperm adhesion molecule 1 (Spam1 or PH-20) is synthesized by the epididymal epithelium, preferentially in the distal region, and is released into the luminal fluid. We also showed that whereas testicular and epididymal Spam1 have hyaluronidase activity at neutral pH, they are under different transcriptional regulation. The aim of this study was to further compare characteristics of the two forms of this glycosyl-phosphatidylinositol-linked protein and their transcripts, and to determine whether secreted epididymal Spam1 is released with its lipid anchor. With GeneRacer amplification of the 3' end of the complementary DNA we show that the poly(A) tails are significantly ($P < .05$) shorter in the epididymis than in the testis. Two-dimensional polyacrylamide gel electrophoresis with immunoblotting reveals one to three isoforms for epididymal Spam1 with the isoelectric point (pI) ranging from 7.3 to 9.0, and four isoforms ranging from 6.6 to 9.0 pI for testicular Spam1. Two isoforms with a pI ranging from 7.6 to 9.0 were observed for caudal sperm. Lectin blotting analysis shows that *Phaseolus vulgaris* erythroagglutinin, *Lycopersicon esculentum* lectin (LEL), and *Solanum tuberosum* lectin, which all bind to N-linked chains, recognize a 67 kd band in the epididymis and caudal sperm, but not in the testis. Treatment of the protein extracts with anti-Spam1 serum prior to blotting with LEL led to the disappearance of the banding, indicating Spam1 specificity of the staining. The lectin peanut agglutinin, which preferentially binds to O-linked side chains, recognizes a 67 kd band in all three cell types. Enzymatic deglycosylation studies confirmed the presence of an O-linked glycan in all three cell types. Ultracentrifugation of the luminal fluid reveals that epididymal Spam1 is secreted predominantly as insoluble particles, which when treated with phosphatidylinositol-specific phospholipase C or Triton X-100, reveal that the majority of epididymal Spam1 is released with its lipid anchor, a form in which it can bind to sperm.

Key words: GPI-anchor, insoluble particles, epididymosomes

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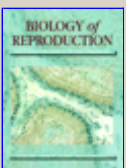
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