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Binding of Protein D/E to the Surface of Rat Epididymal Sperm Before Ejaculation and After Deposition in the Female Reproductive Tract

CHRISTOPHER E. TUBBS^{*,†,§}, JOSEPH C. HALL^{‡,§}, ROBERT O. SCOTT^{‡,§},
VICTOR P. CLARK^{‡,§}, TONIA L. HERMON^{‡,§} AND CARTHENE BAZEMORE-WALKER^{‡,§}

From the ^{*} Department of Biochemistry, North Carolina State University, Raleigh, North Carolina; [‡] Departments of Biology and Chemistry, Norfolk State University, Norfolk, Virginia; [§] Laboratory for the Study of Reproductive Biochemistry and Molecular Biology, Innovative Reproductive Technologies, Virginia Beach, Virginia.

[†] Present address: Department of Genetics, Cell Biology & Development, University of Minnesota, 321 Church St. SE/4-135 Jackson, Minneapolis, MN 55455-0217.

Correspondence to: Joseph C. Hall, Department of Chemistry, Norfolk State University, Norfolk, VA (e-mail: jhall@nsu.edu).

The objectives of the present investigation were to study the interaction of protein D/E with the surface of rat epididymal spermatozoa and to assess its topology on the spermatozoa surface before and after deposition in the female reproductive tract. Protein D/E, a member of the cysteine-rich secretory protein (CRISP-1) family, has been proposed to be involved in sperm-egg membrane fusion. In vitro competitive photoactivated cross-linking experiments followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis revealed that protein D/E molecules specifically interact with two surface proteins exhibiting an M_r ~120.0 kd and ~130.0 kd, respectively, on the sperm surface. In vitro treatment of epididymal spermatozoa with phosphatidylinositol specific-phospholipase C revealed the release of protein D/E molecules over the head region but not the tail region of spermatozoa. Indirect immunofluorescence experiments using polyclonal antibodies generated against a highly purified protein D/E preparation demonstrated that protein D/E molecules were bound to the surface of spermatozoa recovered from the epididymal and female reproductive tracts, even after 7 hours. These results indicate that protein D/E molecules interact with specific membrane proteins, and is subsequently covalently bound to the surface of spermatozoa via a glycosyl-phosphatidyl inositol linkage. In addition, protein D/E molecules remain covalently bound to spermatozoa after deposition in the female reproductive tract, an observation that is consistent with the proposed physiological function of the protein in the fertilization process.

Key words: Epididymal secretory protein, interaction, sperm plasma membrane

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