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JOURNAL ARTICLE

# Importance of glycosylation and disulfide bonds in hyaluronidase activity of macaque sperm surface PH-20

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PH-20 is a glycoprotein located on the surface of the sperm plasma membrane and on the inner acrosomal membrane. The best understood function of sperm surface PH-20 is its hyaluronidase activity, which results in hydrolysis of the hyaluronic acid-rich cumulus matrix during sperm penetration of this extracellular oocyte investment. In

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this study, we investigated whether alterations in the secondary and tertiary structures of sperm surface PH-20 would affect its enzyme activity. Proteins were isolated from the sperm plasma membrane by treatment of living cells with phosphatidylinositol-specific phospholipase C (PI-PLC). PH-20 was purified from the PI-PLC released proteins by immunoaffinity chromatography. Twodimensional electrophoresis of purified PH-20 revealed 6 isoforms with isoelectric points ranging from 5.1 to 6.0. Removal of the N-linked glycans from PH-20 with N-glycosidase F shifted the molecular weight from 64 kd to approximately 54 kd, its deduced molecular weight based on sequence analysis, suggesting that most if not all, of the potential N-glycosylation sites are linked to oligosaccharides. The lectins Con A and PSA recognized purified sperm surface PH-20 after Western blotting, suggesting that mannose is a major sugar within or at the terminal end of the linked glycan. The lectins UEA and LPA did not recognize PH-20 Western blot, suggesting that fucose and sialic acid are not terminal sugars of sperm surface PH-20. Deglycosylation of sperm surface PH-20 resulted in a complete loss of its hyaluronidase activity. The reduction of disulfide bonds with beta-mercaptoethanol or dithiothreitol also resulted in loss of enzyme activity. We conclude that the hyaluronidase activity of sperm surface PH-20 is dependent on structural features established by sulfhydryl linkages, as well as glycosylation.

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