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# The Proacrosin Binding Protein, sp32, Is Tyrosine Phosphorylated During Capacitation of Pig Sperm

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Mammalian sperm must undergo capacitation, a preparation period in the female reproductive tract or in vitro, in order to fertilize. We have previously described a Mr 32 000 tyrosine phosphorylated protein, "p32," that appears in pig sperm during capacitation. The identity of p32 remains unknown; if and how it is involved during capacitation is not understood. The objective of the present study was to identify p32 by proteomic techniques. Western blotting of proteins separated successively under nonreducing and then reducing conditions showed the appearance of the tyrosine phosphorylated p32 only when sperm were incubated in capacitating conditions. The spot was sequenced by mass spectrometry/mass spectrometry and identified as "sp32," a protein implicated in proacrosin maturation. The same membranes probed with anti-sp32 antibody demonstrated that sp32 is present in both noncapacitating and capacitating conditions and revealed exactly the same spot as p32. Immunoprecipitation with either anti-phosphotyrosine or anti-sp32 antibody corroborated these results. Indirect immunofluorescence with anti-phosphotyrosine antibody or anti-sp32 antibody show similar labeling of capacitated sperm, supporting the hypothesis that p32 is a tyrosine phosphorylated form of sp32. After ionophore treatment to induce the acrosome reaction, anti-sp32 and anti-phosphotyrosine labeling on the acrosome disappeared. These results demonstrate that sp32, a (pro)acrosin binding protein, is the p32, a tyrosine phosphorylated protein related to capacitation. We will now focus on the significance of tyrosine phosphorylation on sp32 function during fertilization-related events.

Key words: Fertilization, p32, acrosin, acrosome reaction

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