BIOCHEMICAL LETTERS

PH-20 and sperm hyaluronidase: a conceptual conundrum in mammalian fertilization

Hyaluronidase has long been recognized as a quantitatively important enzyme within mammalian sperm acrosomes, but its role in fertilization has remained unproven. The extracellular matrix of the cumulus surrounding the mammalian egg is rich in hyaluronan, and it was long supposed that hyaluronidase assisted the spermatozoa to penetrate this mass. However, hyaluronidase is thought to be released from the fertilizing spermatozoon only at the zona surface, when the spermatozoon exocytoses its acrosome in response to a zona signal. Recently, Gmachl and Kreil (1993) have discovered that PH-20, a glucosylphosphoinositide-anchored sperm surface and acrosomal membrane protein involved in sperm binding to the zona pellucida (Primakoff et al., 1985; Myles et al., 1987), has sequence similarity to bee venom hyaluronidase. Moreover, rabbit kidney cells transfected with recombinant human sperm PH-20 DNA express surface-bound hyaluronidase activity that can be released in a much more active soluble form with phosphoinositide-specific phospholipase C (Gmachl et al., 1993). These surprising results have reopened the debate regarding a role for hyaluronidase activity in fertilization.

We have reached a similar conclusion in relating PH-20 to hyaluronidase, by comparing the N-terminal sequence of sperm hyaluronidase itself with published PH-20 sequence data. Ovine sperm hyaluronidase, which had been purified to homogeneity (119000 W.H.O. units/mg of protein) (Harrison, 1988a,b), was run on an SDS/polyacrylamide gel and transferred to an Immobilon-P membrane by using a semi-dry electroblotting procedure (Matsudaira, 1987). The N-terminal sequence was determined by automated Edman degradation using an Applied Biosystems model 475A gas-phase sequenator equipped with a model 120A phenylthiohydantoin (PTH) analyser for the on-line identification and determination of PTH derivatives. Comparison of the ovine hyaluronidase amino acid sequence with the derived protein sequences of PH-20 cDNA clones from human, monkey and guinea pig (Lin et al., 1993) reveals significant similarity; there is 59% identity between the N-termini of ovine hyaluronidase and the PH-20s from human and monkey (Figure 1). Also, we have identified well-conserved hyaluronan-binding motifs (Yang et al., 1994) in all three PH-20 cDNA sequences (Figure 2).

Nevertheless, these results raise perplexing issues. Despite their apparently similar catalytic activity and their shared N-terminal sequence, PH-20 and the component conventionally recognised as sperm hyaluronidase have curiously different characteristics. Sperm hyaluronidase is essentially a soluble enzyme (Harrison, 1988a) that lies within the acrosomal matrix (Gould and Bernstein, 1975) and is readily released at the time of the acrosome reaction (Triana et al., 1980; Shams-Borhan and Harrison, 1981), whereas PH-20 (in guinea-pig sperm) is essentially membrane-bound, is located to the inner acrosomal membrane during the acrosome reaction, and cannot be detected in the matrix (Cowan et al., 1986). Immunocytochemical studies at the light-microscope level show hyaluronidase only in the anterior regions of the sperm head and not in the equatorial or post-acrosomal regions (Gould and Bernstein, 1975; Harrison and Gaunt, 1988), whereas a significant proportion of PH-20 is located in these latter regions in guinea-pig sperm prior to the acrosome reaction (Cowan et al., 1986). Ovine hyaluronidase from ejaculated sperm has a molecular mass of at least 81 kDa (Harrison, 1988b), compared with an estimate of 48 kDa for PH-20 (Phelps et al., 1990).

A hyaluronan-binding or degrading role for PH-20 during fertilization is not easily envisaged, for the antigen appears to be involved primarily in the binding of acrosome-reacted spermatozoa to the zona pellucida (Myles et al., 1987). High concentrations of PH-20 would indeed be exposed on the anterior sperm head surface as a result of the acrosome reaction, and interaction of the hyaluronan-binding sites in PH-20 (Figure 2) with hyaluronan fibres embedded within the zona (Talbot, 1984) might well enable sperm attachment to the zona; however, the soluble acrosomal hyaluronidase (with a tendency to bind to surfaces; see Harrison, 1988a) would have been released into the same environment, and would be expected to compete effectively with PH-20 for this hyaluronan. It is possible that PH-20 located on the posterior surface of the head of acrosome-intact spermatozoa might assist these cells to penetrate the cumulus matrix by degrading the hyaluronan fibres in the matrix. But the posterior location and membrane-bound nature of the PH-20 does not appear ideal for such a role (a binding and therefore retarding action could equally well be proposed!); more persuasively, motile spermatozoa from non-mammalian animal species lacking hyaluronidase have been shown to pass through the mammalian cumulus readily to reach the egg (Talbot et al., 1985).

Ovine	Xaa-Phe-Arg-Ala-Pro-Pro-Leu-Ile-Ser-Xaa-Thr-Leu-Phe-Leu-Cys-Ala-Ser-Asn-Lys-
Human	Leu-Asn-***-***-***-***-***-Val-***-Pro-Asn-Val-Ala-***-***-Trp-***-Trp-***-Ala-
Monkey	Asn-***-***-***-***-***-Ile-***-Pro-Asn-Val-Ala-***-***-Trp-***-Trp-***-Ala-
Guinea pig	Asp-Lys-***-***-***-***-***-***-Pro-Asn-Val-Pro-Leu-***-Trp-Val-Trp-***-Ala-

Figure 1 Comparison of N-terminal sequences for ovine hyaluronidase and the deduced sequences for recombinant PH-20s from human, monkey and guinea pig

The putative signal peptides have been removed from the cDNA-derived sequences. Xaa represents an undetermined amino acid residue, and *** represents a residue that is identical with the corresponding position in the ovine sequence.

Human/Monkey	¹⁵⁶ Arg-Pro-Thr-Trp-Ala-Arg-Asn-Trp-Lys ¹⁵⁸
Guinea pig	¹⁴⁹ Arg-Pro-Thr-Trp-Thr-Arg-Asn-Trp-Arg ¹⁵⁷

Figure 2 Putative hyaluronan-binding motifs in recombinant PH-20s

The idealized domain is two basic amino acids separated by seven amino acids, of which none should be acidic, but at least one should be basic.

Finally, immunization of ewes against sperm hyaluronidase failed to block pregnancy, despite high antibody titres and inhibition of enzyme activity (Morton and McAnulty, 1979). Antisera to PH-20, on the other hand, block fertilization effectively in the guinea pig, yet do not cross-react with other tissues that express at least one form of hyaluronidase (Primakoff et al., 1988).

Clearly our results and those of Gmachl et al. (1993) have raised a number of fundamental questions, not only about the roles of PH-20 and hyaluronidase in mammalian fertilization, but also about the relationship between PH-20 and the several forms of hyaluronidase identified previously in somatic as well as germ-line tissue.

Peter GACESA,*† Nicola D. CIVILL* and Robin A. P. HARRISON†

*Department of Biochemistry, University of Wales, Cardiff, P.O. Box 903, Cardiff CF1 1ST,

U.K., and †Department of Development and Signalling, The Babraham Institute, Babraham, Cambridge CB2 4AT, U.K.

‡ To whom correspondence should be addressed.

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