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PRESENCE OF β-ASPARTYL *N*-ACETYL GLUCOSAMINE AMIDO HYDROLASE IN MAMMALIAN SPERMATOZOA

V. K. BHALLA, W. L. TILLMAN AND W. L. WILLIAMS

Department of Biochemistry, University of Georgia, Athens, Georgia 30602, *U.S.A.*

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The concept of sperm acrosomes as modified lysosomes which evolved to facilitate fertilization in multicellular organisms has recently been proposed (Allison & Hartree, 1968, 1970). The following lysosomal enzymes have been found in sperm acrosomes: hyaluronidase and neuraminidase (Hartree & Srivastava, 1965; Srivastava, Adams & Hartree, 1965; Srivastava, Zaneveld & Williams, 1970), β-*N*-acetyl glucosaminidase (Conchie & Mann, 1957), acid phosphatase, phospholipase A and aryl sulphatase (Allison & Hartree, 1970), aryl amidase (Meizel & Colham, 1972) and acrosomal proteinases (Stambaugh & Buckley, 1968, 1969; Zaneveld, Srivastava & Williams, 1969; Zaneveld, Polakoski & Williams, 1972; Polakoski, Zaneveld & Williams, 1972). This paper concerns a lysosomal enzyme which cleaves the linkage between aspartate and *N*-acetyl glucosamine using the synthetic substrate, 2-acetamido-1-(L-asparta-mido) 1,2 dideoxy glucose (Cyclo Chemical Corp., Box 71557, Los Angeles, Calif.). The enzyme, β-aspartyl *N*-acetyl glucosamine amido hydrolase (aspartyl amidase), has been shown to be present in the acrosomal extracts of boar spermatozoa (Bhalla, 1972; W. L. Tillman, V. K. Bhalla and W. L. Williams, unpublished observations). The purpose of this communication is to report the presence of this enzyme in other mammalian spermatozoa.

Ejaculates were collected by electroejaculation from squirrel-monkeys of proven fertility. Boar and ram ejaculates were collected by gloved hand and by electroejaculation, respectively. Human ejaculates were donated by males of 20 to 30 years of age. Only spermatozoa showing normal motility were used for the preparation of extracts. Acrosomal extracts were prepared by the method of Hartree & Srivastava (1965), omitting ethanol precipitation. After detergent treatment, the supernatant solution was dialysed against 0·01 M-sodium phosphate buffer, pH 7·6.

The assay mixture for aspartyl amidase contained 100 μg substrate, 0·2 M-phosphate buffer, pH 7·6, and enzyme in a total volume of 0·45 ml. Incubations were performed at 37° C for 1 hr to 4 hr. The reaction was stopped by adding 100 μl 0·8 M-borate buffer, pH 9·1, followed by immediate heating at 100° C for 30 min. The enzymatically liberated *N*-acetyl glucosamine was estimated by the method of Reissig, Strominger & LeLoir (1955). 'Minus enzyme' and 'minus substrate' controls were used in all experiments. No non-enzymatic

decomposition of substrate occurred. A unit of β-aspartyl *N*-acetyl glucosamine amido hydrolase as originally proposed by Mahadevan & Tappel (1967) is that amount required to release 1 nmol N-acetyl glucosamine in 1 hr. The proteins were measured by the method of Lowry, Rosebrough, Farr & Randall (1951), using crystalline bovine serum albumin (Pentex) as standard.

Table 1. Aspartyl amidase activities in semen fractions of various species

Species	*Volume of ejaculate (ml)*	*Seminal plasma*		*Final KRP washings*		*Acrosomal extract*	
		Specific activity	*Total units/ ejaculate*	*Specific activity*	*Total units/ ejaculate*	*Specific activity*	*Total units/ ejaculate*
Boar	320	3·1	16,000	0	0	73	9200
Squirrel-monkey	1	29·6	18	0	0	89·1	4·5
Human	6	4·04	384	3·6	1·4	47·8	14·6
Ram	1	7·3	131	204	102	145	249

KRP = Krebs–Ringer phosphate.

From the results shown in Table 1 for one ejaculate of each species, it is evident that the specific activity of the acrosomal extracts is greater than that of the seminal plasma. A greater amount of enzyme activity per ejaculate was present in the acrosomal extract of ram and boar. No appreciable difference was observed if cytoplasmic droplets were removed by differential centrifugation before preparing the acrosomal extracts by the method of Allison & Hartree (1970). However, the total units of aspartyl amidase were greater in the seminal plasma of boar, squirrel-monkey and human than in their respective acrosomal extracts. The high enzyme activity in the seminal plasma of these three species is consistent with the findings of Allison & Hartree (1970), who have reported

Table 2. Summary of purification of aspartyl amidase from human acrosomal extract

Purification step	*Total units*	*Specific activity (units/mg protein)*	*Fold purified*	*Recovery (%)*
Acrosomal extract	36	48	1	100
Phosphocellulose	23	114	2·5	65
Sephadex G-100	6	1437	30	16

the presence of a large proportion of most of the acrosomal enzymes in the seminal plasma. On the basis of the high specific activity of aspartyl amidase in the acrosomal extract, it is suggested that this enzyme is of acrosomal origin. The higher content of aspartyl amidase in the acrosomal extract of ram spermatozoa compared to ram seminal plasma further supports this suggestion.

Preliminary results on the purification of the aspartyl amidase from human acrosomal extract by phosphocellulose and G-100 Sephadex chromatography are shown in Table 2. The aspartyl amidase was purified 30-fold, with a final specific activity of 1437 units/mg. A linear increase in rate of hydrolysis occurred

with time and increases in enzyme concentration. The pH optimum was found to be 7·8. The enzyme displayed maximum activity at the unusually high temperature of 70° C. The physiological significance of the high temperature optimum and the biological function of this new enzyme in fertilization are unknown at present.

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