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Ovum-Membrane Denudation Activities of the Purified Acrosomal Glycosidases and Sulfatase

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= **국문초록** =

정제된 아크로솜의 글라이코시데이스들과 썰파테이스들의 난자막 침투의 활성도

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양 철 학

정자 아크로좀이나 정액중에서 고순도로 정제된 탄수화물 분해효소들 및 썰파테이스를 개별적으로 또는 여러 조합으로 사용하여 난자막의 용해를 조사하였다.

베타 굴룩유로니데이스와 베타-엔-에시틸글루코사미니데이스가 전혀 없이 정제된 황소 정자의 하이얄유로니데이스는 토끼의 난자막중 큐물러스층을 쉽게 분해시켰다. 토끼의 정자에서 얻은 아릴썰파테이스와 위의 하이얄유로니데이스는 더욱 빨리 토끼난자의 큐물러스층을 용해시켰다.

한편 아릴썰파테이스는 하이얄유로니데이스 활성도를 크게 촉진시키지는 못하였다.

정제된 베타-글룩유로니데이스나 베타-엔-에시틸 글루코사미니데이스들도 하이얄 유로니데이스들과 아릴썰파테이스들처럼 큐물러스층을 용해시켰으나 모든 경우에 난자의 코로나층이나 조나 펠루시다층에는 아무런 변화를 주지 않고 있다.

INTRODUCTION

Sperm penetration into the ovum involves several different enzymatic reactions as sperm acrosomes contain several hydrolytic enzymes. Hyaluronidase is the first enzyme of established physiological function in fertilization (McClean and Rowland, 1972; Austin, 1948; Chang, 1950). Hyaluronidase is localized in sperm acrosomes (Masaki and Hartree, 1950) together with other enzymes, including arysulfatase, β-N-acetylglucosaminidase (Allison and Hartree, 1979) and β-glucuronidase (Dott and Dingle 1968; Bergenstein and Teichman, 1973).

Yang and Srivastava (1974b) suggested that acrosomal arylsulfatases in combination with other sperm acrosomal enzymes may facilitate sperm penetration of the ovum since the cumulus, the corona, and the zona pellucida of the ovum posses sulfated mucopolysaccharides

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(Braden, 1952; Seshachar and Bagga, 1963). Moricard and Gothie (1955) and Gothie (1958) observed marked accumulation of S^{35} between the corona cells and the zona pellucida in the mature follicle of the rabbit oocytes. Sulfated mucopolysaccharides have also been detected in the liquid from cortical granules of sea urchin eggs (Immers, 1961).

No report has been made to test ovum penetration using highly purified hyaluronidase and other enzymes individually or in combination.

MATERIALS AND METHODS

Materials: Freshly super ovulated ova were obtained from female rabbit tracts by flushing with 2 ml of 0.05 M Tris-HCl buffer, pH 7.6.

Methods: The hyaluronidase used in the test was the active fraction obtained from the first Sephadex G-75 step prepared by the method described earlier (Yang and Srivastava, 1974a). Arylsulfatase was prepared as described by Yang & Srivastava (1974b). β-Glucuronidase from bull sperm was purified by $(NH4)_2\ SO_4$ fractionation, two DEAE - cellulose Columns, isoelectric focusing (pH 4-6) and gel filtration on Sephadex G-200. The detailed method for the preparation of enzyme will be described in the future publication. The enzyme used in this test was the active fraction after the isoelectric focusing column. β-N-acetyl glucosaminidase was prepared as described earlier (Yang, 1974c). The enzyme used in the test was obtained after the second gel filtration.

Enzyme Assays: Hyaluronidase activity was determined colorimetrically using hyaluronic acid as substrate in 0.02 M sodium acetate buffer (pH 3.8) containing 0.4 M NaCl (Yang and Srivastava, 1974d). Arylsulfatase was assayed by the modification of the method of Roy (1960) as described earlier (Yang and Srivastava, 1974b). β-Glucuronidase activity was determined by a modification of the method of Fishman et al. (9148) as described by Yang (1981). β-N-acetylglucosaminidase activity was determined by a modification of the method of Tarentino and Maley (1973) as described earlier (Yang, 1974).

Tests of Ovum-Denudation activities: The cumulus clots were collected in a watch glass. The ova in the clot were transfered to 1 ml beakers in a total volume of 0.2 ml containing at least 4 ova per beaker. The beakers were covered with parafilm to eliminate evaporation and incubated at room temperature or 37° in a dry air oven. The ova were observed at intervals with an Olympus inverted microscope at 50x and 100x magnifications. In order to quantitate the cumulus cell dispersing activity the following scale was used:

0 : All ova in cumulus
1 : Cumulus released, corona intact.
2 : Cumulus released, some corona removed.
3 : Cumulus released, all corona removed.
4 : Cumulus released, all corona removed, zona removed.

RESULTS AND DISCUSSION

The Table 1 shows the cumulus removing activity of highly purified hyaluronidase from bull sperm and arylsulfatase from rabbit serpm and arylsulfatase from rabbit sperm acrosomal extracts. The beakers containing 0.3 ml of 0.05M Tris-HCl buffer (pH 6.4), 0.15 M NaCl and 0.1 ml enzyme, were kept at room temperature. Hyaluronidase (1) and Hyaluronidase-Arylsulfatase mixture (IV, V) completely dispersed the cumulus cells within 4 minutes. Allison and Hartree (1970) suggested that arylsulfatase might affect the mumulus cells.

Table 1. Effects of Hyaluronidase and Arylsulfatase on Removal of Cumulus Cells of Rabbit Ova

Enzymes	Units added per assay	Incubation time (min)						
		5	10	15	30	45	60	120
1. Hyaluronidase	0.8	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
2. Arylsulfatase	0.1	1 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (1) 1 (2)
3. Arylsulfatase	0.8	1 (1) 0 (5)	1 (1)) (5)	1 (1) 0 (5)	1 (2) 0 (4)	1 (6)	1 (6)	1 (6)
4. Hyaluronidase & Arylsulfatase	0.8 0.8	1 (2)	1 (2)	1 (2)	1 (3)	1 (3)	1 (3)	1 (3)
5. Hyaluronidase & Arylsulfatase	0.9 1.9	1 (2)	1 (2)	1 (2)	1 (3)	1 (3)	1 (3)	1 (3)
6. Control	–	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)

Number in ( ) indicates number of ova used.

Table 2. Effect of β-glucuronidase and β-N-acetylglucosaminidase on Removal of Cumulus cell of Rabbit Ova

Enzyme & Control	Units added per assay	Incubation time (min)				
		5	15	60	120	24 hr.
1. Control (Distilled water)	–	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
2. β-N-acetylglucosaminidase	3,163 (12.5 μg)*	1 (4)	1 (4)	1 (4)	1 (4)	1 (4)
3. Control (Tris-glycine buffer)	–	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
4. β-glucuronidase	0.97 U (62.3 μg)*	0 (2)	1 (2)	1 (2)	1 (2)	3 (2)

( ) indicates number of ova. *protein

As shown in the results, arylsulfatase removed the cumulus at a slower rate compared to hyaluronidase. Arylsulfatase tested in this experiment was not contaminated with hyaluronidase and β-glucuronidase. Arylsulfatase did not significantly augment the activity of hyaluronidase.

Table II shows the effect of purified β-glucuronidase and β-N-acetylglucosaminidase. Two controls were run one in distilled water and the other in 0.05 M Tris-glycine buffer pH 8.3 as β-glucuronidase was in the buffer and β-N-acetylglucosaminidase was dissolved in glass distilled water. After 24 hours incubation, both controls had intact cumulus but β-N-acetylglucosaminidase removed the cumulus without affecting the corona and zona pellucida. β-glucuronidase removed both the cumulus and the corona radiata. β-Glucuronidase tested in this study had a small amount of Azocoll proteinase activity but no acrosin, hyaluronidase or β-N-acetylglucosaminidase activity. Commercial β-glucuronidase (Worthington Biochemical Co.) did not affect the corona radiata as reported by

Tillman (1972). As reported earlier (Yang, 1981), sperm β-glucuronidase is an unique enzyme compared to other mammalian β-glucuronidases. Further investigation on the specificity of β-glucuronidase is required.

β-N-acetylglucosaminidase was also suggested as a cumulus dispersing enzyme by Allison and Hartree (1970). An interesting coincidence is that the glycosidases studied in these experiments are mostly present in seminal plasma as reported by *Yang (1974C)*. It is suggested that these enzymes might possibly have another physiological function during the fertilization process in dispersing mucoid material in the female tract thereby promoting transport of spermatozoa. It should again be stressed that these are preliminary results and need to be confirmed.

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