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Release of Hyaluronidase and Arylsulfatase from Rabbit Sperm Acrosomes into Seminal Plasma

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—국문초록—

하이얄유로니데이스 및 아릴썰파테이스의 토끼 정자 아크로좀으로부터 정액으로의 배출

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정자의 아크로좀에 존재한다고 알려진 효소들중 실온에 방치할 경우 또는 완충용액으로 정자를 세척할 경우 쉽게 아크로좀 막으로부터 흘러나옴을 관찰하였다. 신선하게 사용된 토끼의 정액을 0℃에서 트리스 완충용액에 방치할 경우 하이얄유로니데이스는 39%정도가 유출되어 나오고, 또 37℃에서 1시간 더 둘 경우 나머지 30%, 그리고 두시간째에 17%가 방출되며, 계면활성제로 아크롬을 처리할 경우 단 4%의 효소가 추출되었다.

다른 두 탄수화물 분해요소인 베타 엔 애시틸글루코사미니데이스 및 베타 글룩유로니데이스도 하이얄유로니데이스처럼 쉽게 분리되어 나오고 계면활성제로 추출될 수 있는 양은 대단히 적었다.

이와같이 효소가 아크로좀에서 방출되는 정도에 따라 아크로좀막에 흡착된 정도를 알 수 있으며 동시에 수정과정에서 역활을 이해하는데 도움이 될 것이다.

INTRODUCTION

Since Swyer (1947) observed the release of hyaluronidase from rabbit sperm into seminal plasma, several investigators have reported on release of the enzyme (Lewis and Ketchel, 1972a) and the relationship between the enzyme release and capacitation (Austin, 1960; Lewis and Ketchel, 1972b; Rogers and Morton, 1973). Hyaluronidase is known to originate from the seminiferous epithelium in rabbit (Swyer, 1947) and in man (Bergenstal and Scott, 1948), and is associated with the spermatozoa. Masaki and Hartree (1962) reported that hyaluronidase occurred in the sperm heads and diffused from the cells to the seminal plasma when stored at 20°. Most of hyaluronidase, arylsulfatase, β-glucuronidase and β-N-acetylglucosaminidase were relased from sperm into seminal plasma when stored at -20°C (Kim and Yang, 1981;

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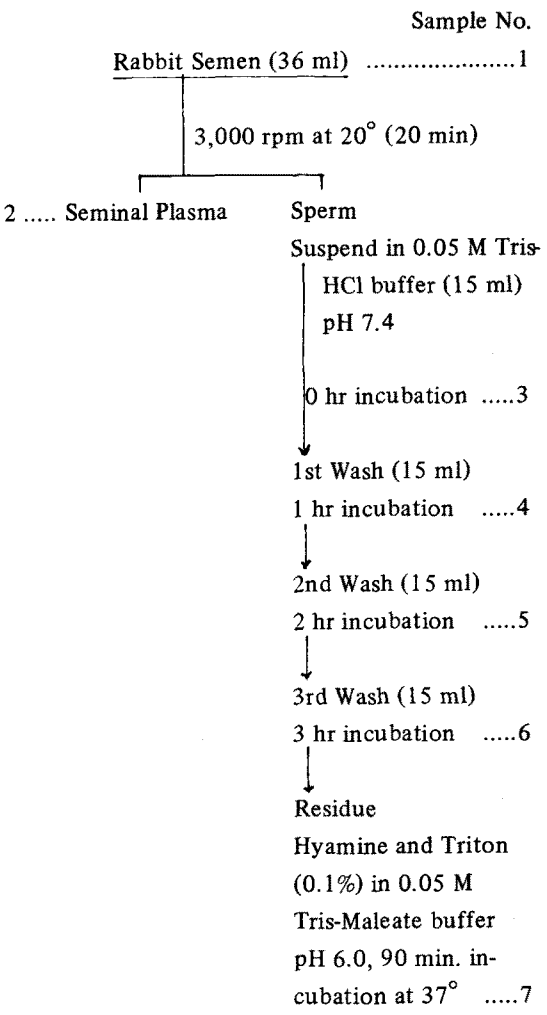
Suh and Yang, 1981). Stambaugh and Buckley (1969) observed that hyaluronidase is associated with acrosin in sperm acrosomes. Capacitation caused release of hyaluronidase through change in the properties of acrosome (Austin, 1960). Lewis and Ketchel (1972a) found that postovulatory uterine fluid appeared to promote the release of hyaluronidase from spermatozoa. Rogers and Morton (1973) reported that the percentage of mobility of spermatozoa was related to the amount of hyaluronidase released in the medium when spermatozoa were incubated in Tyrode's solution.

This paper reports experiments designed to identify other enzymes released from sperm into seminal plasma and medium in vitro along with hyaluronidase. Three enzymes, β-N-acetylglucosaminidase, arylsulfatase (Allison and Hartree, 1970) and β-glucuronidase (Dott and Dingle, 1968) were known to be present in sperm acrosomal preparations.

MATERIALS AND METHODS

Freshly ejaculated rabbit semen was used for these experiments. The procedure to check the release of enzyme is diagrammed in Fig. 1. The incubation was carried out at 37° in a shaking water bath. The first centrifugation at 3,000 rpm separated seminal plasma from sperm and the second centrifugation separated enzymes diffused from sperm cells into medium, 0.05 M Tris-HCl buffer (pH 7.4). After 3 hrs incubation, sperm were treated with Hyamine (0.1 %) and Triton (0.1 %) in 0.05 M Tris-maleate buffer (pH 6.0) to obtain the release of membrane bound enzymes from sperm acrosome (Srivastava et al., 1970). Hyaluronidase activity was measured colorimetrically as described earlier (Yang and Srivastava, 1974). Arylsulfatase activity was determined by the modified method of Roy (1960) using 15 mM nitrocatechol sulfate as substrate (pH 6.0). β-N-acetylglucosaminidase and β-glucuronidase were assayed by the method of Tarentino and Maley (1972) and Fishman et al. (1948) respectively.

Fig. 1.



RESULTS AND DISCUSSION

The results based on two replications are summarized in Table I. These results are considered in two categories:

(1) *The release of enzymes as a result of centrifugation to remove sperm and seminal plasma fractions.* A comparison of column 1

Table 1. Release of Enzymes from Rabbit Spermatozoa

Sample No.	Vol.	Hyaluronidase			Aryl sulfatase			β-N-acetylglucos-aminidase			β-glucuronidase		
		U/ml	T.U.	%	U/ml	T.U.	%	U/ml	T.U.	%	U/ml	T.U.	%
1. Semen	36	4	144	–	5	180	–	208	7,488	–	ND	–	–
2. Seminal Plasma	30	22	660	–	10	300	–	695	20,850	–	3	90	–
3. 0 hr.	15	69	1,035	39	19	285	17	152	2,280	20	1	15	45
4. 1 hr.	16	50	800	30	19	304	18	398	6,368	55	0.1	2	6
5. 2 hr.	16	28	488	17	5	80	5	97	1,552	13	0	0	0
6. 3 hr.	16	16	256	10	2	32	2	42	672	6	0	0	0
7. H & T	16	6	96	4	61	976	58	49	784	7	1	16	48

ND = Not determined.

and 2 shows that the centrifugation alone released these enzymes into seminal plasma as amounts of the enzymes found in seminal plasma are higher than in the whole semen.

(2) *The release of enzymes from* sperm free from seminal plasma as a *results of standing and on final treatment of sperm with detergent* it is assumed that a sum of enzyme units in steps 3 through 7 represents the total enzyme present in the sperm fraction. The release of enzymes into the medium at each step is, therefore, given as percent of the total. On this basis approximately 39 % of hyaluronidase is released by holding sperm in hypotonic Tris-buffer at 0°C, 30 % after 1 hr standing at 37°C, 17 % after the second hour, 10 % after the third hour and only 4 % hyaluronidase was extractable by detergents. It is apparent, therefore, that most hyaluronidase is easily released and easily leaks out from spermatozoa.

For arylsulfatase, only a small amount of the enzyme was released into the seminal plasma although 40 % of the enzyme leaked into hypotonic buffer washings with successive incubation. About 60 % of the total enzyme remained in spermatozoa and was released by detergent treatment with Hyamine and Triton.

Some β-N-acetylglucosaminidase activity occurred in the seminal plasma during centrifugation alone, but approximately 75% of the enzyme leaked into the medium within an hour. Only 7 % of the enzyme was extracted by detergent treatment.

Most β-glucuronidase was found in seminal plasma. Half of this enzyme leaked into the hypotonic buffer during centrifugation and rest of the enzyme was extracted by detergent treatment.

These results indicate that each enzyme has a different pattern of release possibly related to their localization in spermatozoa.

Hyaluronidase is known to be involued in the dispersal of the cells of the cumulus oophorus of the ovum by dissolving the intercellular matrix which is composed of hyaluronic acid penetrating the entry of the spermatozoa through this barrier. According to the results obtained in this experiment, hyaluronidase with other glycosidase has been postulated multifunctional. These enzymes might facilitate the sperm passage through mucoid material present in the seminal fluid into female uterus. But arylsulfatase which is tightly bound to acrosomal membrane must have different function during fertilization process.

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