OCCURRENCE OF AMYLO-1,6-GLUCOSIDASE IN HUMAN SEMINAL PLASMA: ITS PROPERTIES AND SIGNIFICANCE IN SPERM METABOLISM

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Summary. The amylo-1,6-glucosidase activity of human seminal plasma was studied using its specific substrate, α -glucosyl Schardinger dextrin. The enzyme activity of semen is predominantly associated with the seminal plasma and proceeds at maximum velocity at pH 5.8 to 6.0. The secretions of seminal vesicles and testis seem to contribute most of the enzyme activity to the seminal plasma. Amylo-1,6-glucosidase activity is significantly lower in azoospermic samples as compared to those with a good sperm count.

INTRODUCTION

Human seminal plasma has been shown to contain amylase (Karassik, 1927) and maltase (Sheth & Rao, 1962a, b). Both these enzymes are known to be involved in glycogen metabolism. Amylo-1,6-glucosidase has been shown to play an important rôle in glycogen degradation. It was, therefore, of interest to find out whether amylo-1,6-glucosidase was present in human semen and whether the level of the enzyme is of metabolic or pathological significance.

MATERIALS AND METHODS

Semen samples were obtained from fertile donors and from persons referred to our laboratory by the infertility clinic of the K.E.M. Hospital. The seminal plasma was separated from spermatozoa by centrifuging the semen at 300 g for 30 min. The plasma was stored at -18°C and used when required.

Amylo-1,6-glucosidase activity was estimated according to the method of Taylor & Whelan (1966). The substrate, Schardinger α -dextrin to which an α -glucose unit is attached in 1,6-linkage, was kindly made available by Dr W. J. Whelan (U.S.A.). It has been demonstrated that amylo-1,6-glucosidase degrades the substrate to form glucose and Schardinger α -dextrin. Amylo-1,6-glucosidase activity of semen was assayed by measuring glucose production using the glucose oxidase method as described previously (Gunaga, Sheth &

Rao, 1967). The enzyme activity was expressed as mg of glucose liberated in 30 min at 37° C per ml of semen.

The fructose content of seminal plasma was assayed according to the paper chromatography method of Sheth & Rao (1959).

RESULTS

Localization of amylo-1,6-glucosidase in the semen

Results depicted in Table 1 indicate that the enzyme activity was localized

TABLE 1
AMYLO-1,6-GLUCOSIDASE LOCALIZATION IN
HUMAN SEMEN

Sample no.	Whole semen	Sperm suspension	Seminal plasma
1	7.7	3.5	7.4
2	36.7	5.2	36.8
3	9.5	2.1	9.5
4	19.3	4.2	20.0

The enzyme activity is expressed as mg of glucose liberated in 30 min at 37° C per ml of semen.

in the seminal plasma. Spermatozoa washed twice with normal saline exhibited low amylo-1,6-glucosidase activity. These results are similar to the observation, that the maltase activity of human semen is contributed predominantly by seminal plasma (Sheth & Rao, 1962a). Enzyme activity of the sperm suspension may be partly derived from the seminal plasma still present in the suspension fluid.

Properties of seminal amylo-1,6-glucosidase

Results, as can be observed from Text-figs. 1, 2 and 3, indicated that the reaction velocity of seminal amylo-1,6-glucosidase was a function of time, pH and enzyme concentration. The reaction proceeded at maximum velocity at pH 5.8 to 6.0. The amount of glucose formed was proportional to the enzyme concentration. The enzyme activity progressively diminished on heating the seminal plasma above 40° C (Table 2).

Buffer

Experiments were performed to find out whether the constituents of the buffer used would influence the pH activity curve. Using a citrate-phosphate buffer, a single peak was obtained with an optimum activity around pH 6.0. A similar curve was obtained with only citrate in the buffer (Text-fig. 2).

The influence of buffers of different ionic strengths on the enzyme activity was also investigated. No change could be observed in the activity when the ionic strength of the buffer ranged between 0.05 and 0.2 M.

Amylo-1,6-glucosidase activity in relation to spermatozoa per ml, motility and volume

The enzyme activity of human semen was studied in relation to percentage

TEXT-FIG. 1. Effect of incubation time on the amylo-1,6-glucosidase activity. The digestion mixture contained 0.08 ml seminal plasma, 0.02 ml of substrate (0.85 mg of dextrin mixture) and 0.1 ml of 0.1M-citrate-0.2M-phosphate buffer pH 5.8.

TEXT-FIG. 2. Effect of pH on the amylo-1,6-glucosidase activity. The digestion mixture contained, 0.08 ml seminal plasma (diluted with buffer to an appropriate protein concentration), 0.02 ml of substrate (0.85 mg of dextrin mixture) and 0.1 ml of 0.1M-citrate-0.2M-phosphate or 0.1M-citrate buffer of desired pH. Incubated at 37° C for 30 min.

motility, spermatozoa per ml and volume. In the twenty-three samples studied, the average value of glucose formed was found to be within a range of 3.4 to 68.6 mg/ml of semen, mean value being 21.4 mg/ml of semen. Amylo-1,6-glucosidase activity was significantly lower in azoospermic samples as compared to those with a good sperm count (Table 3). No correlation was observed

TEXT-FIG. 3. Effect of enzyme concentration on the reaction velocity. The digestion mixture contained 0.08 ml seminal plasma, 0.02 ml of substrate (0.85 mg of dextrin mixture) and 0.1 ml of 0.1M-citrate-0.2M-phosphate buffer pH 5.8. Incubated at 37° C for 30 min.

TABLE 2
HEAT INACTIVATION OF AMYLO-1,6-GLUCOSIDASE

Temperature (°C)	% of original activity
30	100
37	100
50	40.2
60	3.7
70 and above	Nil

between the enzyme activity and percentage motility or volume of the semen sample.

Source of seminal amylo-1,6-glucosidase

It was of interest to find out whether the prostate, seminal vesicle or testis is the source of the enzyme in seminal plasma. Samples of split-ejaculates were obtained from two individuals. It is known that the first portion of the ejaculate consists mostly of prostatic secretion. This is based on the absence of fructose in this portion of the ejaculate. It was possible to obtain three samples of prostatic secretion. The second portion of the split ejaculate consists chiefly of seminal vesicular secretion. This is indicated by the presence of fructose in it (Harvey, 1957). The results, as can be seen from Table 4, indicate that the

major part of the seminal amylo-1,6-glucosidase is contributed by the seminal vesicles and testes. The prostate secretion is unlikely to contribute significant amounts of the enzyme to the semen.

TABLE 3
AMYLO-1,6-GLUCOSIDASE IN RELATION TO SEMEN QUALITY

Type	No. of samples	Amylo-1,6-glucosidase per ejaculate $\pm$ S.E.
1. Azoospermic	7	11.83 $\pm$ 3.2
2. Oligospermic: Sperm count less than 30 million/ml	6	17.97 $\pm$ 6.3
3. Normospermic: Sperm count more than 30 million/ml	10	30.17 $\pm$ 6.01

Between Groups 1 and 2 $P < 0.3$ not significant.

Between Groups 2 and 3 $P < 0.2$ not significant.

Between Groups 1 and 3 $0.02 < P < 0.05$ significant.

The enzyme activity is expressed as mg of glucose liberated in 30 min at 37° C per ml of semen.

TABLE 4
FRUCTOSE VALUES AND AMYLO-1,6-GLUCOSIDASE ACTIVITY OF PROSTATIC SECRETION AND SEMINAL SPLIT EJACULATES

Secretions	Samples	Volume (ml)	Sperm count (million/ml)	Motility (%)	Fructose (mg/100 ml)	Amylo-1,6-glucosidase activity
Prostatic secretion	Mean of 3	—	—	—	Nil	6.94
First portion of split ejaculate (mostly prostatic secretion)	Sample A	0.5	35	55	33.3	7.64
	Sample B	0.6	50	60	24.8	10.41
Second portion of split ejaculate (mostly seminal vesicular secretion)	Sample A	1.2	115	65	300.0	32.63
	Sample B	1.5	110	65	224.4	25.69

The enzyme activity is expressed as mg of glucose liberated in 30 min at 37° C per ml of semen.

DISCUSSION

It is well known that the degradation of glycogen can be effected by any of the following three processes: (a) phosphorylysis by the enzyme phosphorylase; (b) de-branching by transferase and amylo-1,6-glucosidase; (c) hydrolysis by amylase and maltase. Human semen has been shown to contain amylase and maltase in significant amounts. The present investigation has demonstrated the presence of amylo-1,6-glucosidase in human semen. Further, it was observed that the level of the enzyme activity was significantly higher in samples with a good sperm count than in azoospermic and oligospermic samples. The occurrence of all glycogen-degrading enzymes in the semen indicates that glycogen which is present in the male and female tract could provide a substrate for the metabolism of spermatozoa.

Amylo-1,6-glucosidase of human semen was found to have optimum activity at pH 5.8 to 6.0. This optimum is similar to that reported by Taylor & Whelan (1968) for amylo-1,6-glucosidase of rabbit muscle, but considerable disagreement exists as to the true optimum pH of the enzyme as measured with different substrates and by different methods. When dextrin was used as a substrate, the pH optimum was anywhere from 5.0 to 7.4 for the rabbit muscle enzyme (Larner & Schliselfeld, 1956). Taylor & Whelan (1968) have reported that the type of buffer used also influences the shape of the pH activity curves. These workers reported that, in a buffer containing citrate and phosphate, or only phosphate, the enzyme gave a single peak with an optimum at pH 6.0 but, when a buffer containing only citrate was used, a second peak became evident with its mode at pH 7.6. They concluded that there were either two enzymes, or one enzyme with double pH optima, in their material. The present data demonstrate that, irrespective of the type of buffer used, a single peak having an optimum activity at pH 5.8 to 6.0 was observed for the enzyme in human semen.

The activity of seminal amylo-1,6-glucosidase was nearly destroyed when it was heated for 30 min at temperatures above 60° C, whereas seminal maltase activity was relatively stable to heating at 60° C. Only 75% of the activity was destroyed at this temperature (Sheth & Rao, 1962a).

The results of the present investigations have revealed that the amylo-1,6-glucosidase present in human semen was mostly contributed by the seminal vesicular and testicular secretions. The secretions of the prostatic gland and the first portion of a split ejaculate exhibited very low enzyme activity. It was shown earlier by us that seminal maltase, another enzyme which is responsible for the glycogen degradation, was mainly contributed by the prostate.

Recent studies have indicated that both amylo-1,6-glucosidase and maltase activities of human semen increase with androgen therapy (Gunaga, Sheth, Rao & Pardanani, 1969).

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