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Prostatic Origin of Fucosyl Transferase in Human Seminal Plasma – A Study on Healthy Controls and on Men with Infertility or with Prostatic Cancer

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Summary. Fucosyl transferase was recovered in soluble form in human seminal plasma. The enzyme had very little activity, as it was structurally bound to prostasomes, which are membrane-surrounded organelles in seminal plasma. The fucosyl transferase activity was recorded on Sephadex G200 chromatography of seminal plasma (supernatant after ultracentrifugation) in one single peak coinciding with that of prostate-specific acid phosphatase. Studies on healthy men and on men with prostatic cancer suggest a prostatic origin of fucosyl transferase activity; two of the men with prostatic cancer displayed 50–95% decreased activities. Antiandrogenic therapy in another man with cancer resulted in substantial reductions in seminal plasma contents of fucosyl transferase, ATPase, acid phosphatase and fructose suggesting a role of testosterone in their secretions.

Key words: Seminal plasma, Prostasomes, Fucosyl transferase, Infertility, Prostatic cancer, Antiandrogenic therapy.

Introduction

Fucosyl transferase is one of a series of enzymes known as glycosyltransferases that sequentially add specific sugars to a protein or glycoprotein molecule. Their origin and function in a body fluid like blood serum is unknown [15]. Presumably, these transferases are released from secretory cells and from disintegrating cells of various types [2, 6]. It has been speculated that such enzymes may provide a repair mechanism for membrane surface molecules (e.g. erythrocytes) which are subjected to degradation in course of time [23].

In a previous study we reported high fucosyl transferase activity in human seminal plasma [21]. Since the same high activity was found in seminal plasma from vasectomized men, any substantial contribution of this activity from spermatozoa and testicular or epididymal secretions could be ruled out [21]. An origin from other accessory glands was suggested.

The aim of the present investigation was to trace the source of fucosyl transferase in human seminal plasma. Since at least a majority of cellular glycosyltransferases are structurally linked to membranes [5, 10, 20], it was of interest to investigate a possible relationship in human seminal plasma between fucosyltransferase activity and membrane-surrounded organelles of prostatic origin, named prostasomes [19, 24, 25].

Material and Methods

Patients

Total ejaculates were delivered by 34 men subdivided into three groups. Fifteen of them (Group 1) underwent seminal investigations because of infertility. Their mean age was 31 years (range 27–44) and the spermiograms showed variations in sperm contents from 42–468 x 10^6. Morphologically abnormal spermatozoa varied between 25–85% (mean 47.5, ±21.1). Sperm forward progression was subjectively graded and was equal to or greater than 2 (scale of 0–4) in all cases. Penetration test scores according to Nilsson et al. [18] varied between 14–37 mm/h.

Group 2 consisted of 16 men, mean age 37.5 years (range 21–46). They delivered their semen as a follow-up to rule out any presence of spermatozoa after voluntary sterilization by vasectomy. The semen was devoid of spermatozoa in all cases.

Three men with prostatic carcinoma contributed a third group. Patients 1 and 2 had moderately–poorly differentiated carcinomas while the third patient had a well differentiated carcinoma. All three patients delivered their semen prior to treatment with an antiandrogenic drug (Flutamide, 2-methyl-N-[4-nitro-3-(trifluoromethyl) phenyl] propanamide, Schering Corp, New Jersey, USA). The third patient also delivered a semen sample two weeks after the initiation of treatment with this drug.

Fractionated ("split") ejaculates were obtained from 4 other men. Two of them had fathered a child during the last year and displayed normal spermiograms. Two others had infertility problems. Their spermiograms showed 21.4 and 128 x 10^6 sperms (total count), respectively, and 88% and 78% abnormal sperms. These two ejaculates also contained 41% and 80% motile sperms and the mucous sperm penetration ability for top were 10 and 15 mm/h, respectively.

Table 1. Distribution of fucosyl transferase (FT) and sialyl transferase (ST) activities (in cpm/0.2 ml) in supernatant and pellet II after ultracentrifugation of seminal plasma from two categories of men

		Group 1 (n = 15)			Group 2 (n = 16)		
		mean	SD	range	mean	SD	range
Supernatant	FT	24,787 (94.3%)	10,250	14,688–53,908	19,649 (92.8%)	15,821	1,064–63,452
	ST	138 (96.6%)	98	26–264	83 (98.0%)	93	14–298
pellet II	FT	1,493 (5.7%)	580	676–2,564	1,515 (7.2%)	2,402	16–9,048
	ST	4.9 (3.4%)	14.9	0–52	1.7 (2.0%)	2.6	0–7

Per cent of total activity is given in brackets

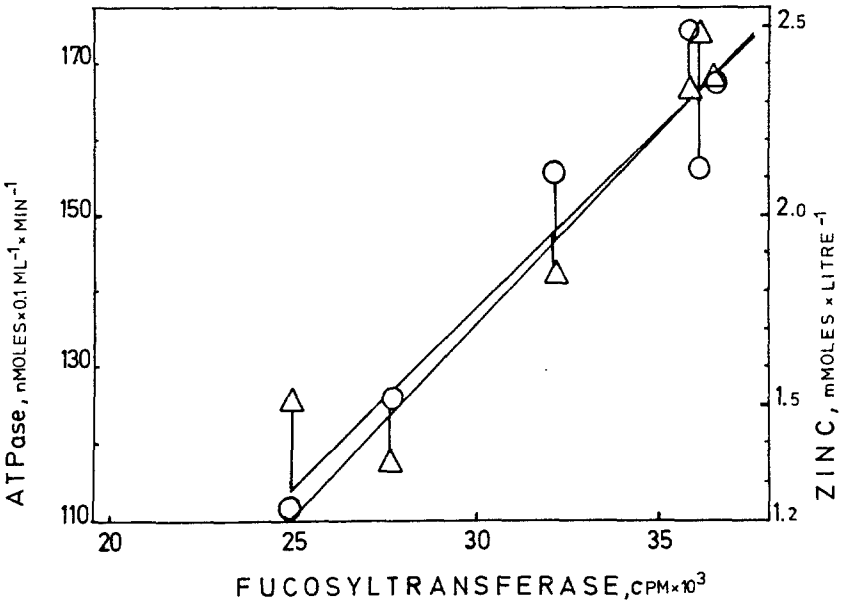

Fig. 1. Linear relationships between fucosyltransferase activity in six different "split" fractions and zinc concentration (*circles*, r = 0.96) respective ATPase activity (*triangles*, r = 0.94). Each plot is connected to its respective line by vertical bar

Biochemical Procedure and Analysis

By a two-step centrifugation procedure spermatozoa and cell debris were separated in pellet I [19]. After preparative ultracentrifugation another supernatant was separated from another pellet (pellet II) [19]. This latter pellet was resuspended in 0.155 mol/l of NaCl to the initial semen volume. The supernatant obtained after ultracentrifugation was in some instances subjected to chromatography on Sephadex G200 in accordance with a procedure described in a previous paper [25]. The material was used for analysis either immediately after preparation or stored at −70 °C until analysis. Sialyl- and fucosyltransferases were determined by the use of an affinity adsorbent technique recently described [21].

Mg^{2+}- and Ca^{2+}-dependent ATPase, fructose and Zn^{2+} were analysed in agreement with a previous investigation [19]. The method of Lowry et al. [16] was employed for protein determination. A competitive-binding, "labelled antigen" technique was used for the isotopic assay of prostatic acid phosphatase taking advantage of a commercial kit available for this procedure [8].

For some experiments (Table 2) the catalytic activity of acid phosphatase was measured in seminal plasma as recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology [26], using paranitrophenyl phosphate as substrate with L(+)-tartrate as inhibitor of acid phosphatases of non-prostatic origin.

Results

The distribution of fucosyltransferase activity between supernatant and pellet II-containing prostasomes after preparative ultracentrifugation is given in Table 1. It is seen that the majority of fucosyltransferase activity was recovered in the supernatant. This was valid for both seminal plasma from patients with fertility problems and from vasectomized men. Since the pellet II was not washed, the small activity monitored (5–7% of total) probably represented that of trapped plasma together with some possible adsorbed, unspecific activity. The same pattern was repeated for sialyl transferase (Table 1), although this activity was on a much

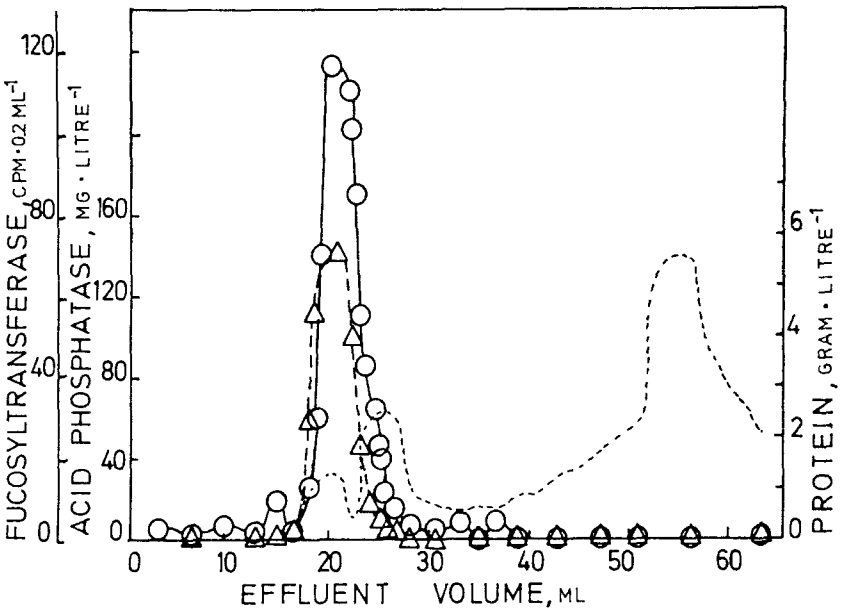

Fig. 2. Sephadex G200 column chromatography of supernatant after ultracentrifugation of seminal plasma from a vasectomized man. Concomitant elution of fucosyltransferase activity (*circles*) and prostatic acid phosphatase, isotopic assay (*triangles*). Protein elution pattern is given by dotted line

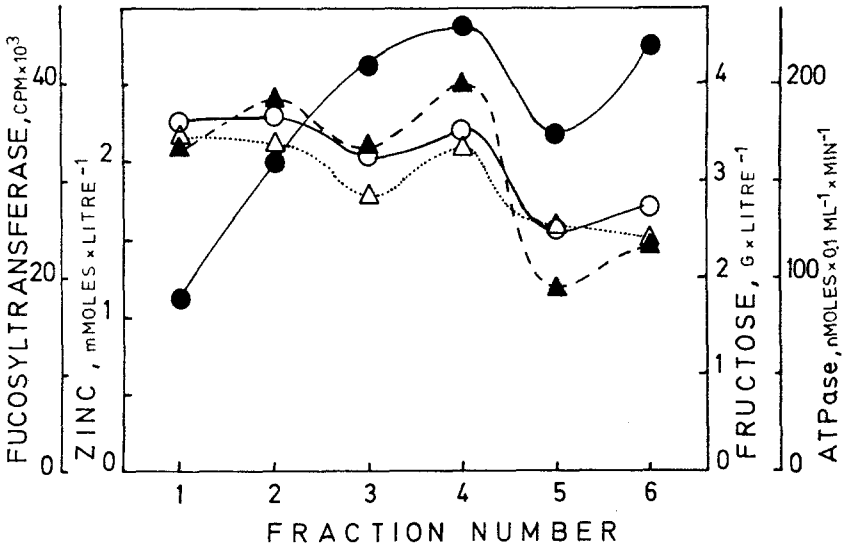

Fig 3. Fucosyltransferase (*open circles*) and ATPase (*open triangles*) activities as well as zinc (*filled triangles*) and fructose (*filled circles*) concentrations in 6 different "split" fractions from a man with well differentiated carcinoma of the prostate gland prior to treatment with Flutamide

lower level than fucosyl transferase activity which confirms a previous investigation [21]. Again, only 2–3% of the total activity was encompassed by the pellet II material from both groups of patients whereas the surplus was found free in supernatant solution (Table 1). "Split" fractions from a total of 4 men were analysed in regard to fucosyl transferase and ATPase activity and Zn^{2+}. A common and consistent pattern was evident in all 4 men. Hence, a linear relationship existed between fucosyltransferase and both ATPase and Zn^{2+} (Fig. 1). These two latter compounds are known prostatic gland markers [9, 14, 19]. An inverse but weak relationship was also noted between fucosyltransferase and fructose ($r = -0.30$, not shown in Fig. 1).

The separation pattern of seminal plasma (supernatant after ultracentrifugation) on Sephadex G200 chromatography from a man with a normal spermiogram is given in Fig. 2. Fucosyltransferase was recovered in the first peak coinciding with prostatic acid phosphatase when identified with regard to antigen sites (RIA method). This first peak also corresponded to a small protein peak while a major protein peak was eluted later (Fig. 2).

Figure 3 illustrates the contents of fucosyl transferase, ATPase, Zn^{2+} and fructose in 6 different "split" fractions from a man with prostatic carcinoma (well differentiated). Comparable curves for fucosyltransferase, ATPase and Zn^{2+} are evident. Fructose displayed a different curve profile. The unusual profile in this case for prostatic markers may be explained by disturbed contractility of the prostate due to the tumour.

Table 2 contains data from 3 patients with prostatic carcinoma prior to treatment with an antiandrogenic drug. In addition, data are given for one of the patients after such

Table 2. Total seminal plasma content of fucosyltransferase activity and of other parameters in 3 men with prostatic carcinoma prior to treatment with an antiandrogenic drug and in 11 men (controls) without prostatic adenoma or carcinoma

	Total volume, µl	Fucosyl transferase, cpm/ejaculate	ATPase, nmol/ ejaculate, min	Acid phosphatase, nkat/ejaculate	Fructose, mg/ejaculate
Patient No 1	269	10.2×10^3	195	319	0.54
Patient No 2	3,755	142.4×10^3	378	13	0.16
Patient No 3	2,600	305.3×10^3	7,502	41,901	5.8
Patient No 3 2 weeks after treatment with Flutamide	1,163	1.6×10^3	1,163	1,368	0.02
Controls					
mean	3,500	281.5×10^3	2,980	34,820	7.32
SD	1,100	295.3×10^3	1,270	38,550	3.37
range	2,000–5,700	$(151.5–1,076) \times 10^3$	1,500–5,880	5,760–63,000	1.80–14.5

treatment. For comparison, data are also given from healthy controls. Two of the patients with moderately to poorly differentiated prostatic carcinomas displayed low or very low values for fucosyl transferase, ATPase and acid phosphatase in their seminal plasma. Fructose content was low as well. Patient number 3 with a well differentiated carcinoma had normal values prior to treatment. A decrease on treatment with Flutamide took place which was most marked for fucosyl transferase and fructose (Table 2).

Discussion

Fucosyl transferases have been detected in soluble form in human milk [11, 22] and as membrane-bound enzymes in hog gastric mucosa [11] human stomach mucosa and submaxillary glands [7], HeLa cells [3] rat intestinal mucosa [1] and pig liver [13]. Our present results strongly support the idea that fucosyl transferase is a soluble secretory product in human seminal plasma, as it is in human milk. The results on "split" ejaculate fractions from different types of patients showing positive correlations to prostatic markers and a negative correlation to fructose suggest a mechanistic link. We therefore conclude that fucosyl transferase is actively secreted by the prostate gland. The existence of a soluble fucosyl transferase in human seminal plasma was also emphasized by the coincidence of the two peaks on Sephadex chromatography, one peak representing this enzyme the other being prostate-specific acid phosphatase. This chromatogram was confirmatory of a previous study with regard to protein distribution [25]. Thus, the two enzymes were eluted early and concomitantly with a minor fraction of proteins while the major protein content of seminal plasma was eluted later.

Our study also demonstrated a 50–95% reduction in seminal plasma content of fucosyltransferase in two of the patients with carcinoma of the prostate gland prior to treatment although the abatement was more pronounced for the other parameters recorded. These two patients had moderately to poorly differentiated carcinomas while the third patient had a well differentiated carcinoma and preserved levels of these prostatic markers. However, antiandrogenic treatment in this third patient induced a 99.5% reduction of the fucosyl transferase activity. Such drastic decreases in seminal plasma parameters were also noted for fructose and acid phosphatase. This reduction was absolute since the diminution in semen volume was comparatively modest. A high degree of reduction was also noted for ATPase in this patient (about 85%). Since the ATPase system is linked to membrane structures surrounding special organelles (prostasomes) [25] these changes suggest a role for testosterone in the secretion of prostasomes as well as acid phosphatase, fucosyltransferase and fructose. Such a proposed role of testosterone is in line with previous reports [4, 17].

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