Comparison Between Proteinases of Human Seminal Plasma and of Sperm Origin

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A comparison of the alkaline proteinase activity of human seminal plasma, the seminal non-gamete cellular material and spermatozoa was made by gelatin-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (gelatin-SDS-PAGE) zymography. Several major (molecular weights = > 56,000) and minor (35,000 to 44,000) bands of proteinase activity were seen in the seminal plasma samples from nonvasectomized and vasectomized, healthy donors. Similar activity profiles were observed in the nongamete cellular material of vasectomized donor ejaculates. The major proteinase activity in sperm extracts was in the 47,000 to 55,000 (proacrosin-acrosin) and 34,000 to 37,000 (sperminogen-spermin) molecular weight ranges. These results suggest that the proacrosin-acrosin and sperminogen-spermin systems are of sperm origin and that there are considerable amounts of larger molecular weight trypsin-like enzymes in the soluble and nongamete cellular material of human seminal plasma.

Key words: seminal plasma, proteinase, proacrosin, human, spermatozoa.

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There are a number of proteinases present in human semen, several of which have been reported to have physiologic significance (reviewed by Morton, 1977; Mann and Lutwack-Mann, 1981). A number of these proteinases are of seminal plasma origin, predominantly from prostatic secretions, while others are associated with the spermatozoa. The relationship of the various proteinases in human seminal plasma and spermatozoa is not clear. This is a particularly difficult problem to understand because spermatozoa have been shown to be capable of absorbing proteins from the seminal plasma (Polakoski and Parrish, 1977) and, conversely, enzymes can be released from the spermatozoa into their environment (Goodpasture et al, 1981). Furthermore, very small quantities of human semen have limited detailed investigations on samples from individual donors.

Recently, we have utilized an ultrasensitive proteinase-detection zymograph to identify and characterize partially the proacrosin-acrosin (Siegel and Polakoski, 1985) and the sperminogen-spermin (Siegel and Polakoski, 1984) systems of human spermatozoa. The present study utilized this gelatin-SDS-PAGE zymography to identify and characterize partially the proteinases in human seminal plasma and seminal nongamete cellular material. This zymographic technique was used to compare seminal

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plasma proteinases to the proteinases of the spermatozoa in individual ejaculates.

Materials and Methods

Unless specified, chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Samples were obtained from nonvasectomized and vasectomized donors by masturbation. Semen samples from nonvasectomized donors were analyzed by light microscopy and the sperm concentration determined using a hemocytometer (Zaneveld and Polakoski, 1976). Samples from vasectomized donors were examined for the presence of spermatozoa and/or nongamete cellular forms. Washed spermatozoa (4×10^6) from semen of nonvasectomized donors and the nongamete cellular fraction from vasectomized donor ejaculates were obtained by centrifugation (4000 rpm for 10 minutes at 4 C) of the samples through 12% (w/v) ficoll (type 400-DL), *p*H 7.6. The soluble seminal plasma fractions and the resulting washed sperm pellets and the nongamete cellular pellets were either treated and used immediately or stored at −20 C. Treatment of spermatozoa and nongamete cellular material consisted of acid extraction (room temperature) in 0.5 M acetic acid containing 0.08% (w/v) sodium azide for 30 minutes. The soluble seminal plasma fraction was treated with the extraction medium. All treated samples were centrifuged at 1200 rpm (Eppendorf 5414) for 10 minutes and the pellets were discarded. Human proacrosin was prepared by a fast protein liquid chromatography system (Siegel et al, 1987). The standard reference proteins and the approximate molecular weights were: phosphorylase b 97,000; BSA 66,000; ovalbumin 45,000; trypsinogen 24,000 and lysozyme 14,000. A 5-μl aliquot of each sample was mixed with an equal volume of SDS-sample treatment buffer (Siegel and Polakoski, 1985). The samples were then applied to gelatin-SDS-PAGE slab gels (Heussen and Dowdle, 1980) and treated as previously described (Siegel and Polakoski, 1985). Following electrophoresis, SDS was removed from the gels by soaking the gels for 30 minutes with Triton X-100 (2.5%, v/v). The Triton X-100 was removed with several washes of distilled water and the gels were incubated for 18 to 20 hours at 37 C in 50 mM Tris(hydroxymethyl)aminomethane + 50 mM $CaCl_2$ (*p*H 8.0). In some cases, the trypsin inhibitor, benzamidine (50 mM), was added to the Triton X-100 wash, the rinse, and to the incubation buffer to determine the trypsin-like specificity of individual digestion bands. The gels were then stained for protein with Coomassie blue (0.125% w/v) and destained with 50% methanol-10% acetic acid. The gels were then restained with Amido black (0.1% w/v) and destained with the methanol-acetic acid buffer. Proteinase activity was detected by observing a clear area of gel on a darkly stained background.

Results

The soluble fraction of seminal plasma from two nonvasectomized donors (Fig. 1, A and B) and ejaculates from three vasectomized donors (Fig. 1, C, D, and E) contain major digestion brands (Fig. 1, lanes A to E) with relative molecular weights of 56,000 or greater. In addition, minor bands (two doublets; designated by arrows) of alkaline proteinase digestion (Fig. 1, lanes A to E) were also apparent with relative molecular weights between 35,000 and

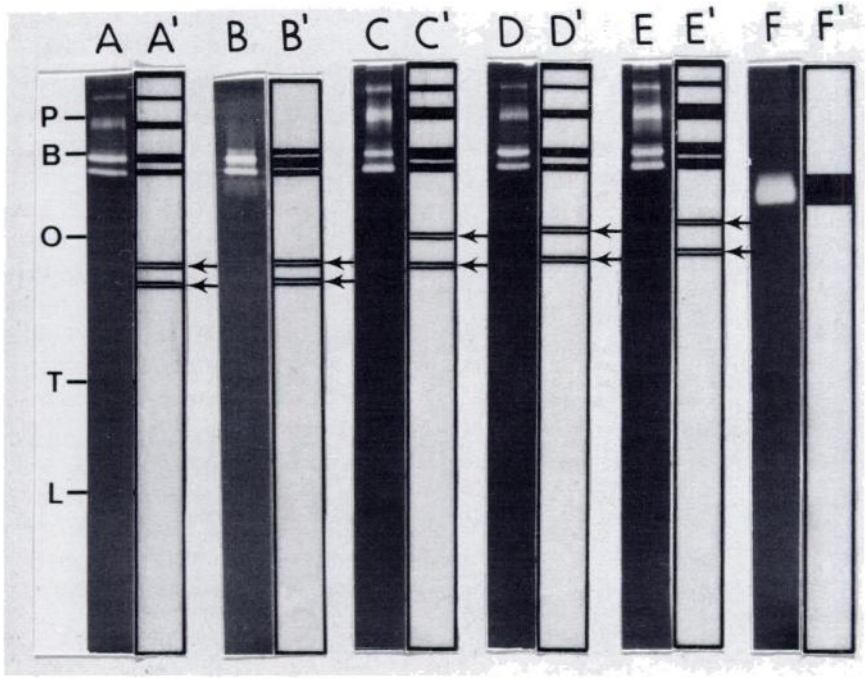

Fig. 1. Alkaline proteinase activity of human seminal plasma as detected with gelatin-SDS-PAGE zymography. Lanes A and B show the proteinase activity resulting from seminal plasma from two individual nonvasectomized donors. Lanes C, D and E demonstrate the proteinases in seminal plasma from three individual vasectomized donors. Lane F contains purified proacrosin. The letters on the left hand margin represent the respective distances that standard proteins migrated into the gels. Arrows indicate minor (doublet) bands of proteinase activity. Corresponding schematics (see prime letters) are presented to indicate activity bands that were partially obscured during the photographic process.

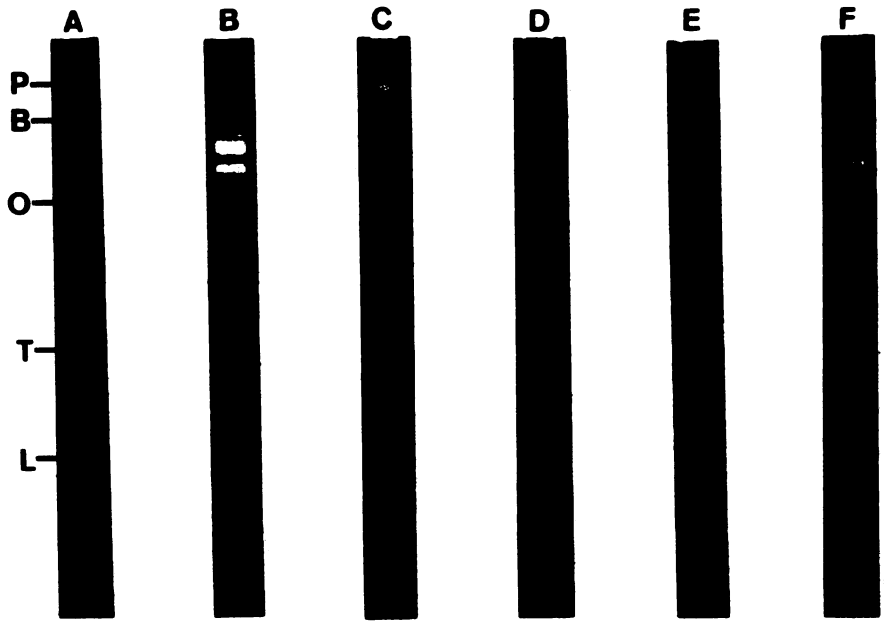

Fig. 2. Alkaline proteinase activity of washed semen pellets. Lanes A and B are the proteinase digestion from the extract of washed sperm pellets of two individual donors. Lanes C, D and E represent the nongamete portion of the pelleted semen samples from three individual vasectomized donors. Lane F and the letters presented on the left hand margin are the same as that presented in Fig. 1.

44,000. Proteinases found in the nongamete cellular material (Fig. 2, lanes C, D, and E) of vasectomized ejaculates yielded digestion bands very similar to those observed in the soluble seminal plasma portion. However, there was noticeably less digestion in the nongamete cellular fraction when compared with the soluble seminal plasma portion and the four minor bands of digestion were not apparent. Figures 1 and 2 are representative of zymographs obtained by electrophoresis of seminal plasma, gamete, and nongamete materials from ejaculates of vasectomized and nonvasectomized donors. The proteolytic digestion profiles (ie, the locations of individual activity bands) for the soluble seminal plasma fractions from both nonvasectomized and vasectomized donors were consistent among individuals (Fig. 1). This consistency was also observed for the proteolytic digestion profiles of the nongamete cellular fraction (Fig. 2). The major proteinase activity observed in the spermatozoa extract was in the 47,000 to 55,000 and the 34,000 to 37,000 ranges, which correspond to the proacrosin-acrosin (Siegel et al, 1986) and sperminogen-spermin (Siegel and Polakoski, 1984; Siegel et al, 1987) proteinase systems, respectively. Contamination of spermatozoa with seminal plasma proteinases (Fig. 2, lanes A and B) and of seminal plasma by proteinases from spermatozoa (Fig. 1, lanes A and B) was evident in several samples from nonvasectomized donors. Benzamidine inhibited all of the proteinase activity observed in the soluble seminal plasma, nongamete cellular material and spermatozoa (data not shown).

Discussion

This study reveals that numerous proteinases in semen can be identified and investigated with a recently devised zymographic gelatin-SDS-PAGE technique (Siegel and Polakoski, 1985). The data demonstrate that there are several alkaline proteinases detected by this method that are present in seminal plasma or associated with the nongamete cellular material and/or spermatozoa.

All the major areas of proteinase activity in the seminal plasma from both the nonvasectomized and vasectomized donors had molecular weights equal to or greater than 56,000 (Fig. 1). However, close inspection of the gels also revealed several minor bands (two doublets) with lower molecular weights. In the vasectomized samples (Fig. 1, lanes C, D, and E), there was no observable proteinase activity detected in the 52,000 to 55,000 range that corresponds to the area of proacrosin. However, some digestion in this area was observed in some seminal plasma samples from whole semen (Fig. 1, lane B), indicating a possible leakage of proacrosin from the spermatozoa in the ejaculate. Interestingly, corresponding areas of sperminogen-spermin digestion (molecular weights

of 30,000 to 34,000) have not been observed in these samples, suggesting that this system is either not released or is present in much smaller amounts than proacrosin.

Current evidence indicates that the proacrosin-acrosin and sperminogen-spermin enzyme systems are associated with the spermatozoa (reviewed by Polakoski and Siegel, 1986). The data presented in lanes A and B of Fig. 2 apparently confirm this conclusion because areas of digestion with correspondingly appropriate molecular weights for these two systems were the major proteinases observed in the extracts of the pelleted material from nonvasectomized donors. Also observed in these extracts were several proteinases with molecular weights equal to or greater than 56,000. However, it is not known if these proteinases originated from the spermatozoa, the seminal plasma, or if they are associated with the nonsperm cellular material. Furthermore, similar proteinase activities were seen in the seminal plasma samples (Fig. 1) as well as that observed in the pelleted nonsperm cellular material from the samples donated by the vasectomized males (Fig. 2, lanes C to E).

Benzamidine, a competitive inhibitor, has been used to inhibit trypsin-like proteinases that hydrolyze arginine and lysine peptide bonds. It is, therefore, surprising that benzamidine inhibited all of the proteinases that were investigated during this study, because several nontrypsin-like human seminal plasma proteinases have been reported (Suominen, 1974; Polakoski et al, 1976). It is possible that the conditions chosen for the described technique are not optimal for these other proteinases or that these enzymes are present in zymogen precursor forms that do not autoactivate under the conditions used. Nevertheless, the results presented demonstrate that there are more trypsin-like proteinases present in semen than was previously believed. Finally, even though there are definite similarities in the patterns of the individual samples, minor variations in the number and intensities of the specific digested bands were easily detected. Consequently, it is expected that this technique will be utilized to investigate further the inter- and intra-sample variation of the seminal proteinases.

The ultra small volume of sample utilized with this technique is a particular advantage, allowing multiple and varied analyses of the characteristically small sample volumes of human semen. In addition, the gelatin-SDS-PAGE technique is currently being used to detect the presence of proteinases present in specific disease states of the male reproductive tract.

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