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Simultaneous Identification of Alpha-L-Fucosidase and Phosphoglucomutase (PGM) Subtyping in Semen Stains

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ABSTRACT: Seminal fluid and stains were analyzed by isoelectric focusing to determine the donor phenotype in the alpha-L-fucosidase (AlFuc) polymorphic system. The enzyme is found in both seminal fluid and spermatazoa. Three common phenotypes exist and can be identified in fluid specimens stored at 4°C for more than a year. Untreated semen specimens display more than eight distinct bands of alpha-L-fucosidase activity with isoelectric points of pH 6.6 and below. Neuraminidase-treated specimens have enhanced banding patterns cathodally with a loss of activity in anodal bands making it easier to phenotype specimens. Semen stains maintained in dehumidified chambers at 25 or 37°C retained activity for at least one month and could be accurately phenotyped. Activity was observed in semen specimens maintained at −20°C in the dried state for a period of one year, whereas a complete loss of activity was observed after two weeks in similar specimens maintained at 25 or 37°C under humid conditions. Of seventy-four semen stains analyzed, two had no apparent activity. Of the remaining seventy-two specimens 56, 32, and 12% were phenotyped as FUC 1-1, FUC 2-1, and FUC 2-2, respectively. Calculated gene frequencies are $FUC^1 = 0.72$ and $FUC^2 = 0.28$. Following analysis of alpha-L-fucosidase, the agarose gel can be chemically developed to reveal the PGM_1 subtyping pattern. The ability to phenotype both systems in semen stains significantly improves the ability of the analyst to individualize this type of physical evidence. The probability of discrimination for these two combined systems is approximately 0.89.

KEYWORDS: forensic science, alpha-L-fucosidase, phosphoglucomutase, genetic typing, semen, isoelectric focusing

The lysosomal enzyme alpha-L-fucosidase (AlFuc) [EC 3.2.1.51] is a polymorphic protein which is important in the metabolism of fucose-containing glycolipids, glycoproteins, and oligosaccharides. The enzyme cleaves the L-fucosyl residue by attacking the alpha glycosidic linkage of its substrate. It has been identified in various tissues and organs such as leucocytes, serum, kidney, liver, lungs, spleen, placenta, muscle, and in purified extracts of urine [*1-4*]. AlFuc activity is also known to be present in human semen with unknown function [*5-7*]. The genetic deficiency of AlFuc results in a fatal disease known as fucosidosis. Genetically deficient individuals accumulate fucose containing sugars, such as glycosphingolipid, intracellularly [*8-11*]. Fucosidosis is transmitted as an autosomal recessive trait. The gene locus for human AlFuc has been assigned to the short arm (*p* segment) of Chromosome 1, near the loci of 6-PGD, PGM_1, Rh, and AK_2 [*12*].

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There are three common phenotypes of the enzyme which can be observed following either starch gel electrophoresis or isoelectric focusing. They are designated FUC 1-1, 2-1, and 2-2. The pattern of isozymes results from two alleles, FUC^1 and FUC^2. There is a third rare allele, FUC^0. At least eight isozymes have been described. Differences in isoelectric point between the various isozymes result, in part, because the acidic forms contain more sialic acid residues than the more neutral forms of the enzyme [*13*].The enzyme polymorphism makes it a useful system for forensic science applications. In a population study by Turner et al. [*2*], an analysis of blood specimens obtained from donors in New York City revealed that black donors had gene frequencies of 0.926 for FUC^1 and 0.074 for FUC^2, whereas Caucasians had frequencies of approximately 0.750 and 0.250, respectively. A population study in Northrhine-Westphalia (Federal Republic of Germany) revealed that the gene frequencies of FUC^1 and FUC^2 were 0.745 and 0.255, respectively: thus the plausability of excluding nonfathers from paternity is calculated as 15.4% [*14*]. Analysis of 157 semen specimens in a Japanese population resulted in values of 0.739 for FUC^1 and 0.261 for FUC^2 [*7*].

Phosphoglucomutase (PGM) analysis for forensic science purposes was first described by Culliford in 1967 [*15*]. The enzyme reversibly catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate in the presence of Mg^{++} and glucose-1,6-diphosphate. The PGM_1 [EC 2.7.5.1] system has proven valuable for both bloodstain and semen stain analysis because of its high concentration, relative stability in the dried state, and high discriminatory potential [*16*]. PGM activity is found in both spermatozoa and in seminal plasma. Conventional starch gel electrophoresis reveals the presence of four anodal bands, a, b, c, and d, which can be used to describe three distinct phenotypes designated as PGM 1, PGM 2-1, and PGM 2. The four bands are a reflection of the alleles of the PGM_1 locus. Analysis of PGM_1 by isoelectric focusing, on the other hand, reveals ten distinct phenotypes, resulting from further resolution of these four bands. The subtypes are referred to as "1+," "1−," "1+1−," "2+," "2−," "2+2−," "1+2+," "1+2−," "1−2+," and "1−2−" [*17*]. PGM subtyping increases the discriminating power from 0.55 with three phenotypes to 0.75 with ten phenotypes [*18,19*].

In this paper we report population frequencies of the two codominant genes FUC^1 and FUC^2 based on semen stain analysis. We also describe an isoelectric focusing method for simultaneously determining the AlFuc phenotype and the PGM_1 subtype in an individual semen specimen. Because semen casework specimens are often present in limited quantity, the capability of performing both analyses simultaneously on the same specimen greatly increases the probability of discrimination.

Materials and Methods

Semen Specimens

Semen specimens were donated by individuals who are under study at the New York Fertility Research Foundation. An aliquot was removed for fertility studies and the remainder was maintained in the liquid state at 4°C for 24 to 48 h. The fluid was then divided into three portions: the first was frozen, the second was used for semen stain preparation and analysis, and the third was maintained at 4°C until analysis for AlFuc phenotype by isoelectric focusing. The approximate time of ejaculation was noted for enzyme stability studies.

Semen Stains

Semen stains were prepared by aliquoting 0.25 mL of ejaculate onto cotton fabric (1 cm^2). The preparation was allowed to air dry and was then maintained either in the dried state, at ambient humidity, or in a humidified chamber for various periods of time up to five weeks. These samples were maintained at three different temperatures: 4, 25, and 37°C. Reference

semen stains, which were provided by Dr. P. Gill, Central Research Establishment, Home Office Forensic Science Service, U.K., and kept frozen for approximately one and one-half years were also analyzed.

Lymphocyte Preparation

Twenty-five millilitres of whole blood were collected from a donor by venipuncture. The lymphocytes were purified using Ficoll Paque (Pharmacia Inc., Piscataway, NJ), and the cells were freeze-thawed and lysed according to the method of Turner et al. [*2*]. Centrifugation at $\times 2500\ g$ for 10 min pelleted the particulate matter. The supernatant was concentrated 25-fold using an Amicon B15 Concentrating Box (Amicon Corp. Lexington, MA) and refrigerated until analyzed.

Neuraminidase Treatment

Ten microlitres of Clostridium neuraminidase (500 units/mL) (Sigma Chemical Co., St. Louis, MO) and 2.5 μL of 10mM citrate-phosphate buffer (pH 4.8) were added to 10 μL of the specimen. After incubation at room temperature for 17 h, the mixture was subjected to isoelectric focusing.

Isoelectric Focusing

Thick (1.0-mm) agarose gels were prepared by addition of 0.4 g of IEF-grade agarose (IsoGel, EEO = 0) (FMC Corporation, Rockland, ME) and 4.8 g of D-sorbitol to 35 mL of deionized-distilled water. The mixture was stirred and heated until the solution clarified. When the temperature decreased to 60°C, 2.5 mL of Ampholine, pH range 5 to 7, (LKB Instruments Inc., Gaithersburg, MD) were added, and the mixture was poured into a 15-cm by 20-cm by 1.0-mm gel mold. The gel was allowed to harden for at least 2 h, but preferably overnight, before use. Wicks were cut from Whatmann No. 7 filter paper and soaked in either 0.05M sulfuric acid (anodal wick) or 1.0M sodium hydroxide (cathodal wick). Ten-microlitre liquid semen specimens or seminal stain extracts, incubated with either 10 μL of Neuraminidase or distilled water were applied to Whatmann No. 3 filter paper strips (3 by 5 mm). These strips were placed 1 cm from the anodal wick. The gel was run for 2 h at a constant power of 3 W. The cooling platform was maintained at a constant temperature of 4°C. Prefocusing the gel before specimen application for 15 min at 1 W did not enhance the focusing results.

Gel Staining

Following electrofocusing, the gel was stained as described by Turner et al [*1*]. Cellulose acetate strips were soaked in 0.1M citrate-phosphate buffer (pH 4.8) containing 0.2 mg/mL of 4-methylumbelliferyl alpha-L-fucoside (Sigma Chemical Co., St. Louis, MO). The strips were applied to the surface of the gel and the plate was incubated at 37°C for 30 to 45 min. Isozyme bands were visualized by placing the gel in a vapor chamber containing 4 to 5 mL of ammonium hydroxide for approximately 30 s to enhance the emitted fluorescence and viewed under longwave ultraviolet light (365 nm).

Isoelectric Point Determinations

The isoelectric points of various isozymes were determined using a surface pH electrode (Biorad Inc., Rockville Centre, NY). Measurements were made at 1.0-cm intervals between anodal and cathodal wicks. After staining and development of the gel pattern, banding positions were related to isoelectric point values.

Alpha-L-Fucosidase Fluoremetric Assay

AlFuc was assayed spectrofluorometrically using 4-methylumbelliferyl-alpha-L-fucopyranoside as substrate (Sigma Chemical Co., St. Louis, MO) according to the procedure of Beratis et al. [*20*]. The reaction mixture consisted of 0.1 mL of 0.78m*M* substrate in 0.1*M* citrate-phosphate buffer, pH 5.7. Varying amounts (10 to 50 μL) of enzyme solution were incubated for 30 min at 25°C and the reaction was stopped with 5.0 mL of 0.25*M* glycine-carbonate buffer, pH 10.0. The Beckman spectrofluorometer was set for excitation at a wavelength of 365 nm and emission at a wavelength of 450 nm to monitor the production of 4-methylumbelliferone. A unit of enzyme activity is defined as the amount of AlFuc needed to hydrolyze 1.0 nanomole of substrate per minute at 25°C.

PGM Reaction Buffer

The PGM reaction buffer consisted of 0.3*M* Trizma base and 0.1*M* magnesium chloride and was adjusted to pH 8.0 with 6*N* hydrochloric acid.

PGM Reaction Mixture

The PGM reaction mixture consisted of the following: 35 mg of glucose-1-phosphate (containing approximately 1% glucose-1-6-diphosphate), 2.0 mg of NADP (sodium salt), 1.7 I.U. of glucose-6-phosphate dehydrogenase, 2.5 mg of 3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyl tetrazolium bromide (MTT), 2.5 mg of phenazine methosulfate (PMS), 10 mL of PGM reaction buffer, and 1% agarose. All chemicals used for PGM visualization were purchased from Sigma Chemical Co. Agarose used for PGM reaction mixture was purchased from Serological Research Institute (Emeryville, CA).

Results

Initial studies of AlFuc polymorphism in semen were performed by horizontal agarose gel electrophoresis. Development of the gel consistently revealed smears rather than discrete bands of activity. Although differences between specimens could be discerned, phenotyping was unrealistic given the poor resolution. A partially purified lymphocyte preparation which had been lysed and analyzed in the same way yielded similar results. Nevertheless, the isoenzymes appeared to exhibit relatively high activity as observed on the developed plate. As a result, we attempted to perform isoelectric focusing of untreated fresh semen specimens using Ampholines having a pH range from 5 to 7. At least 8 distinct bands of activity could be discerned (Fig. 1). Individual semen specimens could be differentiated on the basis of 3 different banding patterns. As reported by Gill and Sutton, neuraminidase pretreatment of semen specimens reduced the complexity of the banding patterns [*6*]. The more acidic isozymes tended to become less distinct, while the more neutral isozyme bands became enhanced. Although this facilitated phenotyping, the three phenotypes could still be easily distinguished even in untreated specimens. The isozyme pI values ranged from approximately 5.0 to 6.7 in untreated specimens. For phenotyping purposes the isozymes with pI values between 5.5 and 6.7 are most important. We used this procedure to phenotype 74 semen specimens. The results are seen in Table 1. Of the 3 phenotypes, FUC 1-1 is found to be the most common (56%) and FUC 2-2 is the rarest (12%). FUC 2-1 is found to be the phenotype in 32% of the specimens tested. Based upon these observations, the gene frequencies of FUC^1 and FUC^2 are calculated to be 0.72 and 0.28, respectively. Of the 74 specimens, 2 had no observable activity by isoelectric focusing. Semen specimens obtained from 3 vasectomized individuals were easily phenotyped, thus confirming the observations of Gill and Sutton [*6*]. These results indicate that AlFuc activity is present in human seminal plasma and indicate that spermatozoa need not be present within the specimens for phenotyping pur-

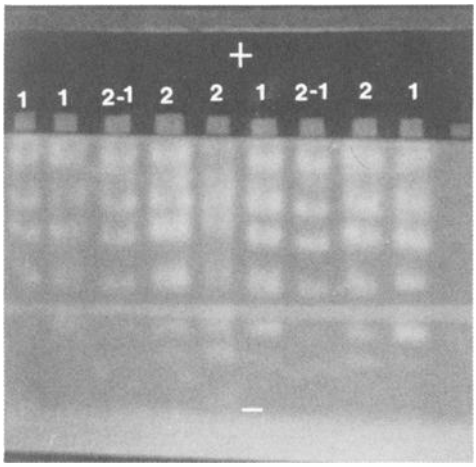

FIG. 1—*Banding pattern resulting from isoelectric focusing of individual human semen specimens illustrating the three major phenotypes of AlFuc.*

TABLE 1—*Results of phenotyping 74 semen specimens for AlFuc; figures in parentheses represent frequencies in percent.*

Phenotype				Gene Frequencies	
FUC 1-1	FUC 2-1	FUC 2-2	Total[a]	FUC^1	FUC^2
40(56)	23(32)	9(12)	72	0.72	0.28

[a]Two specimens displayed no observable activity by isoelectric focusing and could not be phenotyped.

poses. Cotton swabs which had been used to collect vaginal secretions from 3 volunteers were extracted with a minimal volume of distilled water and tested for AlFuc activity. These volunteers indicated that they had not engaged in sexual intercourse during the week before sampling. Despite the fact that all 3 produced results indicating the presence of enzyme activity, the banding patterns were all too weak to be phenotyped.

We have found that immediately after AlFuc phenotyping is completed, the gel can be washed free of AlFuc reaction mixture by gently rinsing with distilled water and analyzed for PGM subtype. This is done by overlaying the gel with PGM reaction mixture containing 1% agarose. After the overlay hardens, the plate is incubated at 37°C for 30 to 45 min and the specimens are then phenotyped for PGM subtype. The four PGM bands, designated "1+," "1−," "2+," and "2−," appear, as expected, in the middle region of the plate with the "1−" band being most cathodal and the "2+" band being most anodal.

AlFuc can be accurately phenotyped in fluid semen specimens kept refrigerated at 4°C or in specimens (liquid or dried) which have been frozen at −20°C for more than one year. The stability of AlFuc in the dried state was determined by preparing semen stains on cotton fabric and maintaining them at various degrees of humidity for varying periods of time up to five weeks and at varying temperatures as described under "Materials and Methods." All such stains maintained at 25 or at 37°C in dehumidified chambers retained activity for at least four weeks. After four weeks, the intensity of the bands began to decrease and accurate typing of some stains became difficult. Stains maintained in a dehumidified chamber at 4°C

are stable for at least six months. Specimens kept frozen at −20°C for approximately one and one-half years could be typed, although the bands were less intense than those observed in fresh stains. Stains maintained at 25 or 37°C under humidified conditions could not be typed at all after the second week of incubation.

AlFuc has been reported to be present in chimpanzee spermatazoa and can be released by extraction with magnesium chloride and Hyamine, and Triton X-100 detergents [*21*]. Results suggested that the enzyme is loosely associated with acrosomal membranes. The acid hydrolases in mammalian spermatozoa are known to be located in the acrosome and they are thought to play a role in fertilization [*22*]. For the site of AlFuc activity in human semen to be determined, three semen samples were pooled and fractionated by centrifugation at ×2500 *g* for 5 min. The supernatant was aspirated off the pellet which consisted primarily of spermatozoa and the pellet was resuspended in isotonic saline and recentrifuged at ×2500 *g* for 5 min; the resulting supernatant was found to be free of intact spermatozoa by light microscopy. The spermatozoal pellet was washed four times with isotonic saline by repeated centrifugation and fractionation as described above. The thoroughly washed spermatozoa were homogenized in a glass/glass hand-held homogenizer in the presence of either Triton X-100 or Hyamine. Alternately, the spermatozoal pellet was suspended in ice-cold barbituric buffer, pH 7.4, placed in an ice bath, and sonicated for a total of 5 min at maximum power using a Biosonik IIA Sonifier (Bronwill Scientific, Rochester, NY) fitted with a microtip. The mixture was recentrifuged at ×2500 *g* for 10 min and the supernatant collected. Whole semen, seminal plasma, spermatozoal homogenate, and the sonicated preparation were tested using the spectrofluorometric assay described in Materials and Methods. Activity was present in the whole semen, seminal plasma, and sonicated spermatozoal preparation. No activity was observed in any of the detergent treated or homogenized specimens. These preliminary studies indicate that AlFuc is associated with both the spermatozoal fraction as well as with the seminal plasma and confirms the observation that ejaculates of vasectomized individuals contains high levels of activity.

We determined the pH optimum using 4-methylumbelliferyl-fucoside as substrate. Three semen specimens were pooled and centrifuged at ×2500 *g* to pellet spermatozoa and the seminal plasma was assayed using the spectrofluorometric assay described in Material and Methods. We found that the enzyme exhibited 2 pH optima. The major peak of enzyme activity was found to center at pH 5.3 and the minor peak at pH 6.3.

Discussion

The identification and individualization of semen is of paramount importance in cases of sexual assault. Various approaches have been described in semen stain analysis. Presumptive testing generally includes analysis of the stain for the presence of spermine, choline, or significantly high levels of acid phosphatase, or some combination of these. Although these substances are characteristic of semen, they are found in other physiological fluids or tissues or both, as well. Confirmatory testing includes the microscopic identification of spermatozoa or the detection within the stain of P30, a protein unique to semen.

Once a stain has been identified as semen, the analyst can begin to individualize it providing that sufficient quantities of stain are available for further analysis. Individualization can be accomplished by an examination of the various polymorphic antigens and enzymes that are present in semen. Forensicially useful polymorphic seminal antigens include the secreted ABH glycoprotein blood group substances and the soluble Lewis a and b antigens. However, forensic science laboratories do not routinely perform tests for Le^a and Le^b in the examination of body fluid stain extracts because of the high cost of anti-Lewis antisera and the reported cross reactivities of the polyclonal antisera available [*23*]. The most useful polymorphic seminal enzyme systems are phosphoglucomutase and peptidase A. Despite the presence of relatively high concentrations of peptidase A in semen, this system is not rou-

tinely used for semen stain analysis because it is not sufficiently polymorphic in the Caucasian population to make it useful in discriminating between individuals [*24*]. Although many other polymorphic enzymes exist in semen, their concentrations either are too low for reliable determinations to be made or they are unstable in the dried state. The technique of DNA polymorphism analysis has been suggested as a useful procedure for analysis of semen stains [*25*]. However, this procedure relies on obtaining a sufficient quantity of spermatozoal DNA for restriction enzyme analysis. It is questionable whether or not this can be often achieved in casework specimens. Furthermore, analysis of seminal stains produced by vasectomized individuals with the latter technique would prove useless since spermatozoa would be absent. As a result, the attempt at individualization is usually restricted to the ABH and PGM_1 genetic systems. The discrimination probability which results from PGM subtyping has been calculated to be approximately 0.75. The use of these two systems simultaneously, brings the discrimination probability to approximately 89%.

In the present study we have demonstrated that PGM_1 and AlFuc, both of which are stable in the dried state for approximately two months, can be simultaneously phenotyped in the same sample, thereby increasing the information available to the analyst with no need for additional specimen. The combined use of the ABH, PGM_1, and AlFuc genetic markers raises the discrimination probability to 96%. Out of 74 specimens studied, 2 had no demonstrable activity. No clinical evidence of fucosidosis exists. A similar observation had been made by Gill and Sutton, who found three infertile individuals lacking AlFuc activity [*6*]. We have no explanation for these observations and cannot establish a definite link between infertility and lack of AlFuc activity, but we feel that these observations merit further study. In the 72 semen specimens successfully phenotyped, we found 56% to be FUC 1-1, 32% to be FUC 2-1, and 12% to be FUC 2-2. The gene frequencies calculated from this data are $FUC^1 = 0.72$ and $FUC^2 = 0.28$ and are in fairly good agreement with previous findings. Gill and Sutton found that the FUC^1 allele has a frequency of 0.704 based upon analysis of 181 semen samples obtained from individuals living in Oxford, U.K. [*6*]. Kido et al. determined that the gene frequency of FUC^1 and FUC^2 are 0.739 and 0.261, respectively, based upon an analysis of 157 semen specimens obtained from Japanese study participants [*7*]. A study of AlFuc gene frequencies in a random population from Rennes, France revealed values of 0.64 and 0.36, respectively [*26*]. A study of 468 individuals from Northrhine-Westphalia (F.R.G.) resulted in values of 0.745 and 0.255 [*14*]. Turner et al. report values of 0.753 and 0.247, respectively, for Caucasians (194 specimens) and 0.926 and 0.074, respectively, for blacks (27 specimens) living in New York City [*2*]. The observations made by the latter three groups were made by analysis of the leukocyte enzyme.

In a study of proteins found in the uterine washings of 21 women, it was determined that alpha-L-fucosidase activity is present and that its activity (which ranges from 0.8 to 9.5 μg *p*-nitrophenol released per milligram protein per hour) is greater than that found in serum [*27*]. Furthermore, AlFuc activity is present in urine [*4*] and in vaginal fluid [*6*] in low quantities. Although this factor can potentially complicate phenotyping of a mixed stain consisting of vaginal secretions and semen, the AlFuc activity of the former is far lower than that of the latter. Neuraminidase-treated specimens display enhanced cathodal banding patterns with decreased activity in anodal bands, thereby making it easier to phenotype samples. Thus, if the female's AlFuc phenotype can be ascertained, it may allow the analyst to reach a conclusion regarding the male's phenotype based upon an analysis of the mixed stain. It is clear that if AlFuc phenotyping of casework is to become forensically useful, a detailed study of such mixed stains must be performed. Such a study should consist of pre- and post-coital specimens obtained from women at various times of the menstrual cycle as well as specimens from post-menopausal and pregnant women.

The use of ioselectric focusing for the analysis of serological evidence has gained in importance over the past few years, primarily because of its detection sensitivity, resolving power,

reproducibility, and speed. It has been used to type erythrocyte acid phosphatase, transferrin, group-specific component, esterase D, alpha 1-antitrypsin, and haptoglobin, and has also been used to subtype PGM_1 [*28*].

The stability of AlFuc in the dried state under various conditions was studied. Kido et al. found that seminal stains could be successfully typed by isoelectric focusing for up to nine weeks when stored at 4°C, for up to seven weeks when stored at room temperature and for up to four weeks when stored at 37°C [*7*]. These observations, as well as our own, indicate that storage temperature is an important factor in enzyme stability and suggest that semen casework specimens should be refrigerated or frozen to preserve activity.

We have found that with the substrate 4-methylumbelliferyl-alpha-L-fucose, human seminal AlFuc exhibits two distinct pH optima. The first at pH 5.3 and the second at pH 6.3. In a previous report, two distinct forms of AlFuc were isolated from human placenta, amniotic fluid, liver, and kidney by gel filtration using Sephadex G-200 [*22*]. These two forms, designated alpha-L-fucosidase I and alpha-L-fucosidase II, were found to have pH optima of 4.5–5.0 and 5.5, respectively, using *p*-nitrophenol alpha-L-fucoside as substrate. In another report, a similar analysis of a purified preparation of human serum AlFuc revealed two pH optima, that is, pH 4.8 and pH 6.1 [*29*]. Brain and liver AlFuc are reported to have broad optima centered around pH 4.7 and 4.6, respectively, with a second minor optima at a more neutral pH of 6.5 and 6.6, respectively. In that study it was determined that the serum enzyme consists of two subunits with molecular weights of 56 500 and 54 500 daltons. It would be of interest to determine if identical subunits are present following purification of the seminal AlFuc. This would provide more information regarding tissue specificity of AlFuc.

The acid hydrolases in mammalian spermatozoa are known to be located in the acrosome and it is thought that they may play a role in fertilization [*30*]. It is possible that various proteases, together with AlFuc present in the seminal plasma, serve to assist sperm penetration of the cervix, which is covered with a mucous containing large amounts of fucose, as suggested by Schumaker [*31*]. The cleaved fucose units that are generated can now be used by spermatozoa for generation of ATP via oxidative phosphorylation. Another possibility is that AlFuc may play a role in the degradation of glycosyl side chains of the zona pellucida glycoproteins and glycosaminoglycans [*21*]. In studies of Limulus polyphemus, the horseshoe crab, it was observed that alpha-L-fucose appears to play an important role in primary sperm attachment to the outer layer of the egg envelope [*32*]. Srivastava et al. studied the distribution of lysosomal hydrolases in sperm acrosomes and seminal plasma in chimpanzee semen samples [*33*]. They extracted sperm with a mixture of Hyamine and Triton X-100 and recovered relatively high levels of AlFuc activity. They were unable to determine with certainty whether the enzyme was bound to the acrosomal membrane (internally or externally) or soluble and within the acrosome. The detergent treatment suggested that the enzyme was weakly bound to the inner acrosomal membrane. The physiological role of human seminal AlFuc in fertilization and reproduction remains unknown.

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