BIOLOGY OF REPRODUCTION 37, 431–439 (1987)

Human Seminal Vesicle-Specific Antigen during Semen Liquefaction[1]

ROBERT S. McGEE and JOHN C. HERR[2]

Department of Anatomy and Cell Biology
University of Virginia Medical Center
Charlottesville, Virginia 22908

ABSTRACT

Binding of monoclonal antibody MHS-5 to western blots of human seminal plasma was employed to follow the fate of a seminal vesicle-specific antigen (SVSA) during semen liquefaction. Ejaculates from four vasectomized donors were collected in a manner to inhibit liquefaction or to allow liquefaction to proceed at room temperature. Aliquots of the liquefying seminal fluid were removed at specific time points and further liquefaction inhibited with sodium dodecyl sulfate (SDS). Western blot analysis using monoclonal antibody MHS-5 demonstrated that the SVSA epitope in all donors was located on multiple bands ranging in mass from 15–92 kDa in unliquefied ejaculates; immunoreactive peptides below 15 kDa were not detected. As early as 5 min post ejaculation, immunoreactive bands below 15 kDA were identified in liquefying samples. During the same time period (5 min), immunoreactive bands of 69–71 and 58 kDa could not be immunologically detected in liquefying samples. A decrease in immunoreactive staining of components higher molecular mass was accompanied by a concomitant increase in immunoreactive staining of intermediate and small molecular mass molecules during the first 2 h of liquefaction. After 8–24 h of liquefaction, two immunoreactive bands of 10.9 and 12.5 kDa predominated. Between 24 and 48 h, each donor's ejaculate demonstrated a common single immunoreactive band of 10.9 kDa. These results indicate that there is a rapid transformation in mass of the SVSA with major 69–71 and 58 kDa bands being converted to forms of lower mass within 5 min of ejaculation.

INTRODUCTION

Seminal fluid of most mammals undergoes coagulation, followed by liquefaction, when the constituents of the male accessory glands are mixed at the time of ejaculation. Coagulation involves the formation of a gelatinous matrix (clot) upon the mixing of vesicular proteins with coagulating gland or prostatic enzyme(s) that catalyze the reaction. Liquefaction is the proteolytic degradation of the matrix, presumably by enzymes also originating from the prostate. The process of coagulation and liquefaction has been studied in the rat (Schon et al., 1982; Williams-Ashman, 1984), guinea pig (Notides and Ashman, 1967), and human (Tauber et al., 1976, 1980; Koren and Lukac, 1979; Lukac and Koren, 1979; Lilja and Laurell, 1984; Lilja and Weiber, 1984; Lilja et al., 1984). Following ejaculation in the human, a soft clot is formed that then dissolves over 5–20 min (Amelar, 1962; Tauber et al., 1980b). Following dissolution of the clot, a post-liquefaction period ensues during which further proteolytic degradation occurs to produce basic, low molecular mass proteins (Lilja and Laurell, 1985).

The protein composition of human seminal fluid has been studied with one- and two-dimensional (1- and 2-D) electrophoresis (Edwards et al., 1981; Leonardi et al., 1983; Rui et al., 1984). Using split-ejaculate analysis, Leonardi and coworkers (1983) have shown that the seminal vesicle contributes basic proteins of low molecular mass (approx. 10–20 kDa) to the ejaculate. Edwards and coworkers (1981), using 2-D gel analysis, have further demonstrated that several high molecular mass protein bands disappear by 30 min post ejaculation, suggesting that liquefaction causes the breakdown of certain seminal proteins. Two enzymes have been proposed to participate in the liquefaction process, collagenase-like peptidase (Lukac and Loren, 1979) and seminin (Tauber et al., 1980). More recently, human prostate-specific antigen (PSA) has been identified as a serine protease (Watt et al., 1986). Seminal vesicle proteins

Accepted March 4, 1987.
Received August 14, 1986.
[1] This work was supported by NIH grant HD-16767 and FBI grant 115744.
[2] Reprint requests: Dr. John C. Herr, Department of Anatomy and Cell Biology, University of Virginia Medical Center, Box 439 Jordan Hall, Charlottesville, VA 22908.

are cleaved upon mixing with prostatic secretions (Lilja, 1985), a finding that led Watt and coworkers (1986) to postulate that PSA may play a role in semen liquefaction.

A monoclonal antibody, MHS-5, has recently been identified that recognizes a sperm-coating antigen secreted by the human seminal vesicle (Herr et al., 1986). Immunocytochemistry of human seminal vesicle tissue localized the MHS-5 antigen to the principal cells of the seminal vesicle epithelium (Evans and Herr, 1986). Cross-reactivity studies against a panel of human tissues and fluids indicated that the monoclonal antibody was specific for the seminal vesicle and the antigen was thus termed seminal vesicle-specific antigen (SVSA; Herr et al., 1986). Due to its specificity for seminal vesicle, the MHS-5 monoclonal antibody has been proposed as a probe for semen identification in sexual assault forensic casework (Herr et al., 1986). The antibody was shown to recognize an epitope located on peptides of a wide molecular mass range when ejaculates were allowed to liquefy for 5 min and analyzed by western blot technique. However, with the same technique, after 15 h of semen liquefaction, immunoreactive peptides of higher molecular mass were undetectable while peptides of lower molecular mass, 8 to 21 kDa, retained antigenicity (Herr et al., 1986). The predominant immunoreactive peptides had molecular masses of 10.0, 11.9, and 13.7 kDa after 15 h of liquefaction.

In view of the literature indicating that vesicular proteins undergo coagulation and subsequent degradation during liquefaction, combined with the observation that immunoreactive peptides containing the MHS-5 epitope were observed to change in molecular weight during liquefaction (Herr et al., 1986), the present study was undertaken to 1) analyze ejaculates from different vasectomized individuals to compare liquefaction-induced changes in molecular weight of SVSA and 2) to conduct an expanded time-course study of transformations in mass of the seminal vesicle-specific antigen during the liquefaction and post-liquefaction periods.

MATERIALS AND METHODS

Monoclonal Antibody Production and Purification

The MHS-5 cell line (IgG_1) was subcloned two times and grown as ascites tumors as previously described (Herr et al., 1985). Balb/c mice (Charles River, Boston, MA) were primed with two i.p. injections of 0.5 ml sterile Pristane (2,6,10,14-tetramethylpentadecane; Sigma Chemical Co., St. Louis, MO) at 2-wk intervals. One week following the second injection, 10^7 hybridoma cells were injected i.p. in 0.5 ml serum-free, sterile RPMI-1640 medium (GIBCO, Grand Island, NY). The ascites fluid was collected and cleared of cellular debris by centrifugation (1,000 × *g*) and stored at −60°C until needed.

The immunoglobulin fraction was precipitated by adding saturated ammonium sulfate drop-by-drop to a final concentration of 40%. The precipitated material was stirred for an additional 3 h at 4°C and then centrifuged at 10,000 rpm (12,000 × *g*) for 15 min. The pellet was resuspended in 1–2 ml H_2O and dialyzed against 0.01 M phosphate-buffered saline (PBS, pH 8.0) with several changes of buffer. Final purification of the monoclonal antibody was achieved by use of a 5-ml protein A-Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) affinity column, as described by Ey et al. (1978). The immunoglobulin fraction was layered directly onto the column at room temperature with a Pasteur pipette and the flow stopped for 2 h. After washing with 12 ml of PBS (pH 8.0), the IgG_1 was eluted with 0.01 M PBS at pH 6.0, the fractions were pooled, concentrated, and stored at 4°C with 0.02% sodium azide as a preservative.

The control antibody used in these experiments was another affinity-purified monoclonal antibody termed Mab-13 (anti-acrosome stabilizing factor, Reynolds and Oliphant, 1984). This antibody is also a murine IgG_1 and does not recognize any components in human semen. It was used at the same antibody concentration as MHS-5 and the nitrocellulose paper processed in the same manner as the experimental nitrocellulose paper.

Collection of Nonliquefied Ejaculates

To inhibit enzymes involved in semen liquefaction and thus obtain ejaculates in a nonliquefied state, vasectomized donors (age 38–50) ejaculated into clean tubes containing 5 ml of 4.0% sodium dodecyl sulfate (SDS; Bio-Rad, Richmond, CA) in double-distilled water. The ejaculates were mixed completely with the SDS immediately after ejaculation and allowed to incubate for 56 h at room temperature. The protein concentration for each of the ejaculates was determined by absorbance at 280 nm. The ejacu-

lates were subsequently diluted to a concentration of 1 mg/ml with sample buffer (0.05 M tris (hydroxymethyl) aminomethane (Tris)-HCl (Sigma Chemical Co.), 30.0% glycerol (Sigma Chemical Co.), 0.2 mM phenylmethylsulfonyl fluoride (PMSF; Sigma Chemical Co.), 1.0% SDS, and 0.01% bromophenol blue (Sigma Chemical Co.; Laemmli, 1970) and boiled for 5 min. β-Mercaptoethanol (Sigma Chemical Co.) was added to a final concentration of 1.0% after boiling. The samples then were subjected to slab gel SDS-polyacrylamide gel electrophoresis (PAGE), which was followed by electrotransfer to nitrocellulose paper (described below).

Time-Course Study of Liquefaction

Two time-course studies were performed to follow the transformation in mass of the SVSA during liquefaction. To obtain liquefied semen, the vasectomized donors, as used in the above study, ejaculated into clean, blank tubes. In one study, 200 μl of liquefied semen was removed from the tube and mixed 1:1 with 4.0% SDS to inhibit further liquefaction at the following time points after ejaculation: 5, 10, 20, 30, 40, 50, 60, and 120 min. In a second time-course study, ejaculates were collected and similarly mixed with SDS at 8, 16, 24, 32, 40, 48, and 56 h. As described above, protein concentrations for each time point in both studies were determined and the samples prepared for SDS-PAGE.

SDS-PAGE and Western Blot Technique

After sample preparation, 20 μg of sample were loaded into each lane of a 15% polyacrylamide gel (16 cm in length), electrophoresed at 10 mA constant-current and subsequently electrotransferred to nitrocellulose paper (Hoeffer, Richmond, CA, 0.2 μm) at 100 mA for 18 h (Towbin et al., 1979). Portions of the nitrocellulose were cut, stained with 0.1% napthol blue-black (Sigma Chemical Co.), dissolved in 10% methanol/10% acetic acid, and destained for approximately 1 h in several changes of 10% methanol/10% acetic acid. Experimental nitrocellulose strips were blocked in 0.01 M PBS (pH 7.2) containing 3.0% nonfat dry milk at room temperature for 30 min and then incubated for 12–18 h at 4°C in either Protein-A-purified MHS-5 antibody or a control monoclonal antibody (Mab-13), both diluted in 0.01 M PBS/0.5% Tween-20 (Sigma Chemical Co.)/1.0% nonfat dry milk to a concentration of 4.0 μg/ml. The strips were washed three times in buffer (PBS/Tween/dry milk) and subsequently incubated with 1/15,000 peroxidase-conjugated goat anti-mouse immunoglobulin (Hyclone, Logan, UT) for 2 h at room temperature. The strips were again washed and antigenic polypeptides were visualized and identified by incubating the strips in 0.04% diaminobenzidine (Sigma Chemical Co.)/0.015% H_2O_2 (Sigma Chemical Co.) in PBS (pH 7.2). Specifically stained bands generally took 5 min to appear and were allowed to develop for an additional 5 min. Staining was terminated by several washes of the nitrocellulose in distilled water. Immunoblots were photographed with a 47B dark blue filter used to enhance contrast. Napthol blue-black-stained nitrocellulose paper was photographed with a R60 red filter.

RESULTS

Ejaculates from three different individuals were collected directly into 4% SDS to inhibit liquefaction or into blank tubes to allow liquefaction to proceed. Ejaculates collected into SDS (0 min of liquefaction) and incubated at room temperature for 56 h were compared to ejaculates allowed to liquefy for 56 h in the absence of SDS. By comparing the 0- and 56-h time points for protein staining and immunoreactive staining, the efficacy of SDS in stabilizing the seminal vesicle-specific antigen was evaluated. If SDS were ineffective in inhibiting liquefaction of SVSA, the expected result would be that the amido-staining pattern and immunoreactive-staining pattern would be similar at time points 0 and 56 h in both SDS-treated and nontreated samples (i.e., liquefaction would proceed normally in SDS). However, in comparing the 0- and 56-h time points for protein staining (Figs. 1 and 3) and immunoreactive staining (Figs. 2 and 4), the patterns for SDS and non-SDS-treated samples were not identical, demonstrating that SDS was effective in stabilizing the seminal vesicle-specific antigen.

When ejaculates collected directly into SDS (0 time point) were compared to those allowed to undergo liquefaction for 5 min to 2 h, the protein composition of the samples changed with time as detected by amido black-staining of the nitrocellulose paper (Fig. 1). Some bands decreased in staining intensity (e.g., 69–71 and 58 kDa) whereas other bands increased in staining intensity (e.g., 15 and 8 kDa). When the nitrocellulose paper containing non-liquefied and liquefied samples was screened with the MHS-5 antibody (Fig. 2), striking similarities were

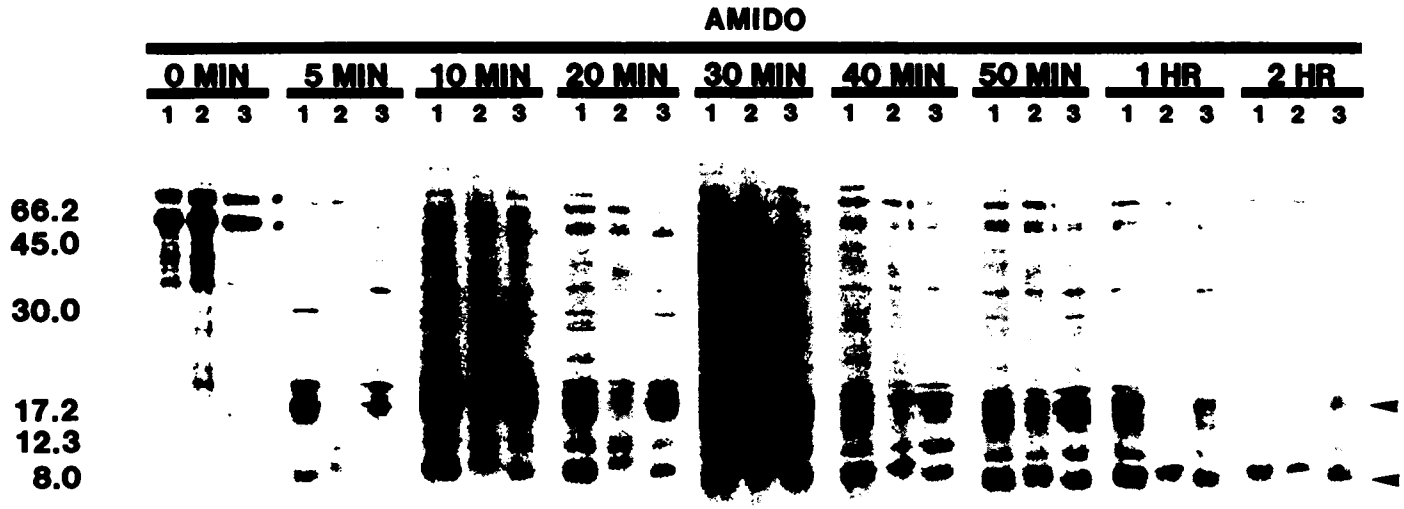

FIG. 1. Western blots of unliquefied and liquefied seminal fluid from three individuals (Donors 1, 2, 3) stained with amido black. Ejaculates were collected directly into sodium dodecyl sulfate (SDS) to inhibit liquefaction of seminal vesicle-specific antigen (SVSA) or allowed to liquefy. Aliquots were removed at designated time points, mixed with SDS and subjected to SDS-PAGE. After electrotransfer to nitrocellulose, the proteins were stained with amido black. In ejaculates collected into SDS to inhibit liquefaction (time point 0), protein bands at 69–71 and 58 kDa appeared as major constituents of unliquefied semen, whereas bands below 15 kDa were not detectable. After 5 min of liquefaction, the protein bands at 69–71 and 58 kDa were observed to decrease in staining intensity (*circles*). There was a concomitant increase in staining of bands 15 and 8 kDa (*arrowheads*) with longer periods of liquefaction.

found in each donor's semen. First, immunoreactive bands of similar relative mobility were found for each individual throughout the time-course study. Each individual demonstrated multiple immunoreactive bands in the unliquefied ejaculates ranging in molecular mass from 15 kDa to 92 kDa. An absence of bands below 15 kDa was a common feature of all ejaculates at 0 time.

By comparison, in liquefied ejaculates, bands with molecular mass less than 15 kDa were observed as

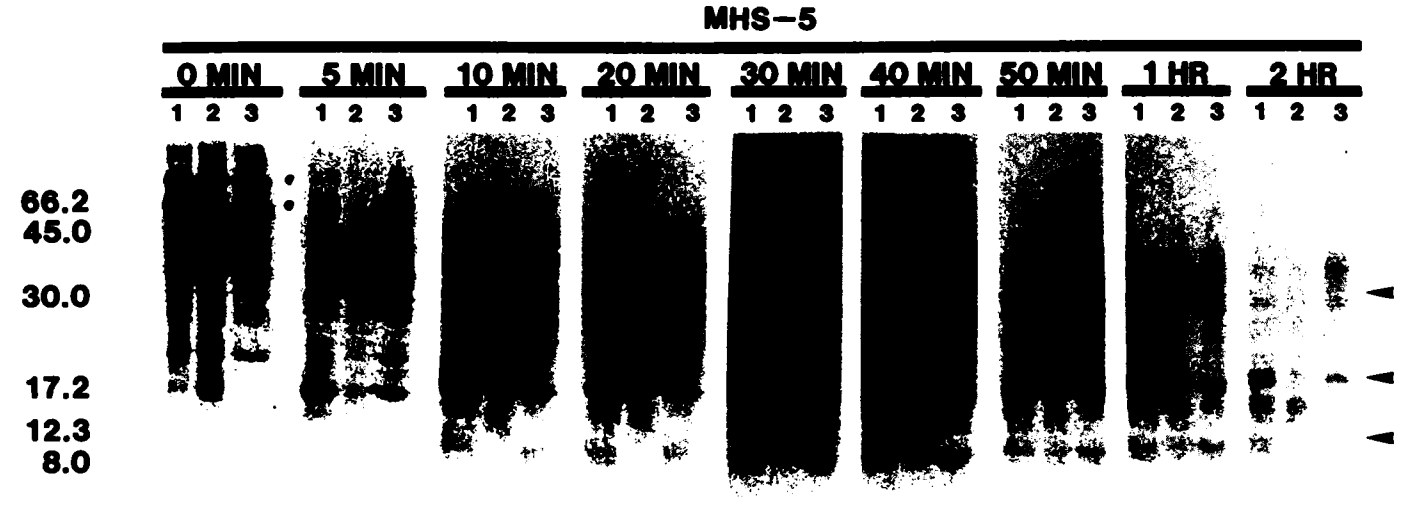

FIG. 2. Immunoblot of unliquefied and liquefied seminal fluid. Ejaculates were collected and processed as described in the *legend* to *Figure 1*. After transfer to nitrocellulose, the samples were screened with the MHS-5 monoclonal antibody. In the unliquefied ejaculates, common multiple immunoreactive bands (15–92 kDa) were detected in each individual, as well as an absence of bands below 15 kDa. Five minutes post-ejaculation, bands with molecular mass less than 15 kDa were observed. Two bands, marked by *circles* (69–71 and 58 kDa) demonstrated a substantial loss in immunoreactivity within 5 min of liquefaction. These two immunoreactive bands correspond to the two major amido-staining bands marked in *Figure 1*. An increase in immunoreactive staining of bands with a molecular mass of 28–31, 15, and 8 kDa was noted (*arrows*).

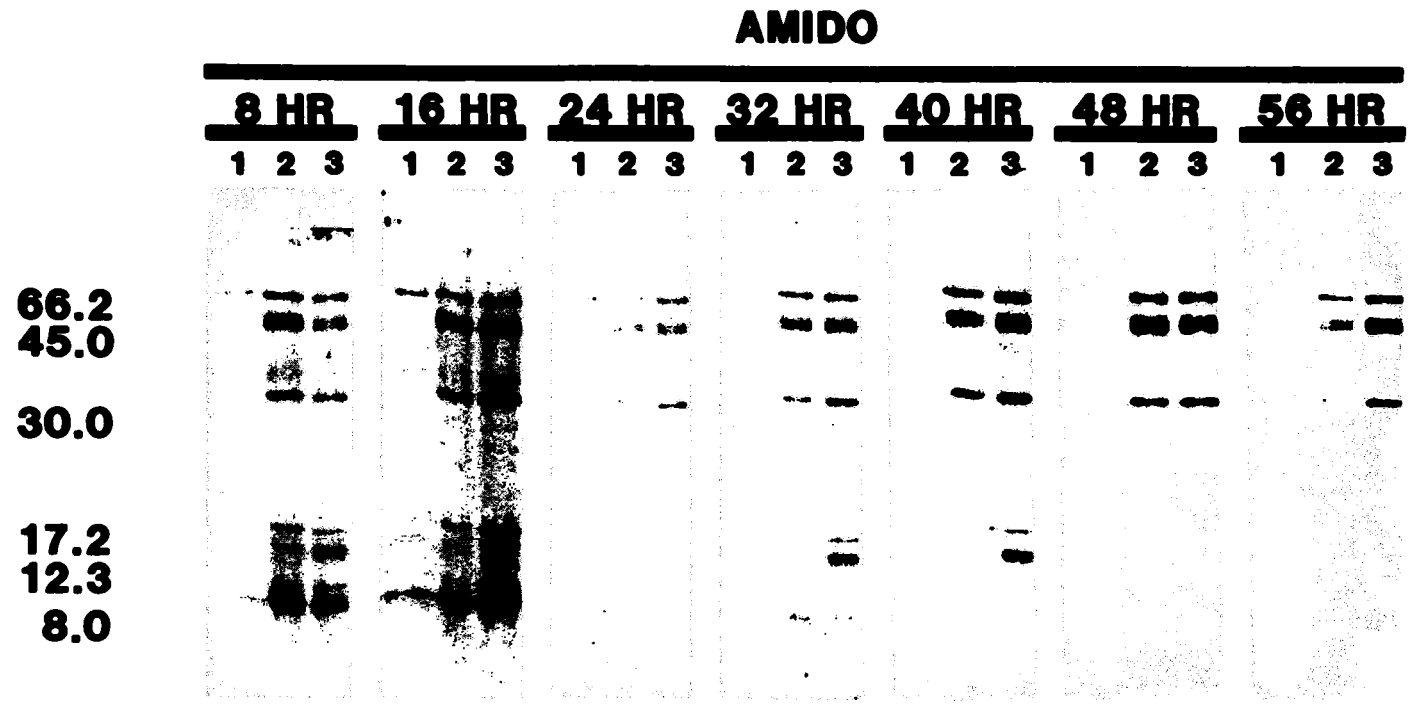

FIG. 3. Western blot of ejaculates from three individuals (Donors 1, 2, 3) liquefied for 8–56 h and stained with amido black. Aliquots of liquefying semen were removed at 8-h intervals, mixed with sodium dodecyl sulfate (SDS) and subjected to SDS-PAGE followed by Western blotting. Several of the higher molecular mass proteins (67, 49, 47, and 35 kDa) remained stable during the 56-h liquefaction period, whereas there was a noticable decrease in staining intensity of the low molecular mass protein (8 kDa) during the same time period.

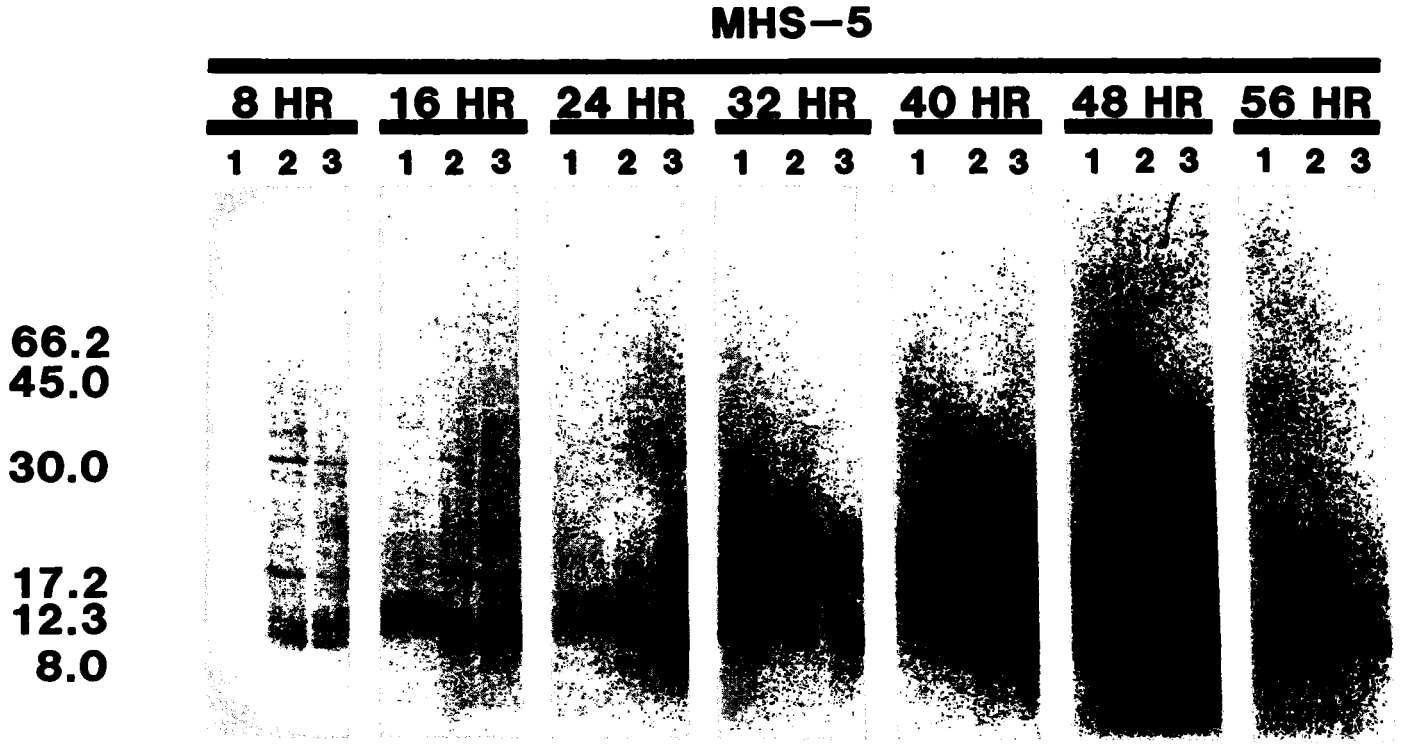

FIG. 4. Immunoblot of ejaculates from three individuals (Donors 1, 2, 3) liquefied for 8–56 h seen in *Figure 3*. The majority of immunostaining occurred below 30 kDa after 8 h of liquefaction. At 16 h post-ejaculation, a triplet of major immunoreactive bands was detected with an apparent molecular mass of 10.9, 12.5, and 13.7 kDa. Between 32 and 48 h, each donor retained the single common immunoreactive band of 10.9 kDa. By 56 h post-ejaculation, only two of the three donors demonstrated an immunoreactive band at 10.9 kDa.

early as 5 min post ejaculation (Fig. 2). These bands below 15 kDa appeared in each individual and increased in immunoreactivity with longer periods of liquefaction (compare 5 min to 10 min). The prominent immunoreactive bands at 69–71 and 58 kDa in unliquefied ejaculates (0 time point) demonstrated a substantial loss in immunoreactivity as early as 5 min post ejaculation and became undetectable after 20 min of liquefaction. The 68–71 and 58 kDa immunoreactive bands corresponded to the two major bands that were observed to disappear during liquefaction on blots stained with amido black.

An increase in immunoreactive staining in certain bands, most notably the bands at 28–31, 15, and 8 kDa, was noted over the 5- to 120-min period (Fig. 2). Bands at 28–31 kDa had little immunoreactivity at 0 min of liquefaction, increased in immunostaining from 5 to 50 min, and then showed reduced immunoreactivity in 1-, 2-, and 8-h samples. Immunoreactive bands with a molecular mass of 15 kDa, while not prominent at 0 time point, showed increased immunoreactivity at 5 min post ejaculation. This increase in immunoreactivity of the 15-kDa molecule continued to 30 min post ejaculation and then declined up to 16 h, whereupon at 24 h of liquefaction, the band could not be detected (see Fig. 4). The 8-kDa immunoreactive band was first detected at 10 min after ejaculation, increased in immunostaining up to 2 h, but was undetectable in samples that had undergone 8 h of liquefaction. It is interesting to note that while amido-stained bands at 8 kDa appear on these gels at the 48-h period, immunoreactivity of the 8-kDa component is lost between 2 and 6 h.

Over the 5- to 120-min time period, there was a decrease in the amount of immunoreactivity in components of higher molecular mass. The decline in immunoreactivity of the 69–71 and 58 kDa components after 5 min has been noted above. In addition, further comparison of 0 min with 5- to 120-min samples showed a progressive loss of other minor immunoreactive bands (e.g., 41.7 and 26.2 kDa).

The results of a 56-h time-course study involving three individuals are shown in Figures 3 and 4. The amido-stained nitrocellulose demonstrated that many seminal proteins of higher molecular mass do not change in their apparent molecular mass during the 8- to 56-h time period (67, 49, 47, and 35 kDa), while some proteins smaller molecular mass, the 8 kDa being the most notable, are less intensely stained after longer liquefaction intervals (Fig. 3). After the nitrocellulose was screened with the MHS-5 anitbody (Fig. 4), the major immunoreactive components were found to be smaller molecular mass species below 30 kDa in semen samples liquefied for 8 h. At 16 h post ejaculation, a doublet of immunoreactive bands (10.9, 12.5 kDa) and a few peptides of higher molecular mass were seen in each individual's ejaculate. At 24 h, this immunoreactive doublet, along with the few higher molecular mass bands, persisted. Between 32 and 48 h, the major immunoreactivity was associated with single (10.9 kDa) or double (10.9 and 12.5 kDa) bands, depending upon the donor's ejaculate. In a sample that had undergone 56 h of liquefaction, immunoreactivity was evident on a common 10.9 kDa component in each donor's ejaculate. The results of this time-course study demonstrate that in each donor's ejaculate the 10.9-kDa component was a relatively stable degradative product of SVSA that appeared initially as part of a doublet of bands, but was retained as a common band upon further liquefaction. The control series of nitrocellulose sheets treated with Mab-13 showed no cross-reactivity at any of the time periods examined (data not shown).

DISCUSSION

The specificity of the MHS-5 monoclonal antibody for a seminal vesicle antigen combined with the sensitivity of western blot analysis provides a novel means of observing changes in mass of a seminal vesicle protein during the liquefaction and post-liquefaction proteolysis of semen. Multiple immunoreactive bands ranging in molecular mass from 15 to 92 kDa were detected in unliquefied ejaculates from each donor, and each did not demonstrate appreciable amido or immunoreactive staining below 15 kDa. As early as 5 min post ejaculation at room temperature, bands below 15 kDa were detectable on blots screened with both amido stain and MHS-5 antibody. Over the 5-min to 2-h period of liquefaction, several immunoreactive species in the higher molecular mass range were not detected. Most noticable was the rapid change in immunoreactivity of the prominent 69–71 and 58 kDa bands during the first 5 min of liquefaction.

The appearance of immunoreactive bands below 15 kDa as early as 5 min post ejaculation suggests a rapid degradative process that induces a transformation in molecular mass of proteins bearing the SVSA epitope. This transformation is best illustrated

by the 69–71 and 58 kDa bands. These bands are major constituents of the unliquefied ejaculate based on amido staining intensity. Five min post ejaculation, these two bands no longer stain as intensely (Fig. 1) as in the 0 time ejaculates. As assessed by immunoblots, these same two bands demonstrated immunoreactivity in the unliquefied ejaculates but significantly lost their immunoreactivity 5 min post ejaculation, an observation which suggests that the epitope recognized by monoclonal antibody MHS-5 was degraded on these proteins (Fig. 2). This loss of higher molecular mass immunoreactive bands (69–71 and 58 kDa) with the concomitant increase in staining intensity of certain smaller molecular mass bands during the 2-h time-course study suggests that the 69–71 and 58 kDa components are the major precursors of the lower molecular mass forms. Proteolytic cleavage of the proteins of higher molecular mass during liquefaction is the most likely mechanism to explain this production of components of intermediate and smaller molecular mass.

A similar transformation in molecular mass of a seminal vesicle component has been observed by Lilja and Laurell (1984, 1985). Prior to in vitro mixing of seminal vesicle and prostatic secretions, a high molecular mass seminal vesicle protein (HMM-SV), with a molecular mass of 76, 71, and 52 kDa following SDS-PAGE (reduced), was detected with a polyclonal antisera (Lilja and Laurell, 1984). Upon incubating prostatic and seminal vesicle secretions for 5 min, four immunoreactive bands immunologically related to HMM-SV were detected below 33 kDa. After 90 min, the majority of immunoreactivity was localized below 20 kDa, with a predominant immunoreactive band at 12.8 kDa (Lilja and Laurell, 1985). The present study has shown that the seminal vesicle-specific antigen identified by monoclonal MHS-5 did not demonstrate immunoreactivity on low molecular mass proteins prior to liquefaction. Low molecular mass, immunoreactive forms appear with increasing intervals of liquefaction, similar to the HMW-SV protein of Lilja and Laurell (1985).

One- and two-dimensional electrophoresis has been used to study the composition of human semen and the effects of liquefaction (Leonardi et al., 1983; Balerna et al., 1984; Rui et al., 1984). These reports demonstrated that vesicular proteins obtained by split ejaculate or glandular massage had a molecular mass less than approximately 25 kDa and possessed basic isoelectric points. Edwards and coworkers (1981), using the BASO-DALT 2-D system to examine the effects of liquefaction of seminal fluid, have also described the disappearance of several proteins during liquefaction. In particular, they found two high molecular mass proteins (66–64 and 30–28 kDa) that were not detectable 10 min following ejaculation and were completely lost by 30 min following semen collection. Three other proteins (series 1–3) were also found to be undetectable within 15 min following ejaculation. The present study further demonstrates such rapid degradative events, in this case involving a specific vesicular protein. The present findings, together with those of previous investigators, emphasize the need to control the time of sample collection and preparation of semen for biochemical analysis.

While coagulation and liquefaction of semen normally occurs within 20–30 min after ejaculation (Amelar, 1962; Tauber et al., 1980b), the results of the present study demonstrate degradation of SVSA proceeds beyond 30 min, with changes in mass of antigenic peptides occurring for many hours following ejaculation. While 20–30 min is descriptive of the time period necessary for dissolution of the coagulum, the post-liquefaction proteolytic events have been given little attention. Lilja and Laurell (1985) found that the HMM-SV protein, which is a constituent of the coagulum, is degraded to a predominant 12.8-kDa protein after 45–90 min of liquefaction. However, by 24 h, the 12.8-kDa protein could no longer be detected on immunoblots, suggesting the initial proteolysis of the coagulum to produce the 12.8-kDa fragment is followed by further proteolysis of the 12.8-kDa peptide.

Seminal vesicle-specific antigen shares several similarities with HMM-SV. The HMM-SV molecule gives bands of 52, 71, and 76 kDa on reduced SDS-PAGE, whereas SVSA shows major immunoreactive bands at 58 and 69–71 kDa. However, in contrast to the reports on the HMM-SV, immunoreactive species of many higher and intermediate molecular mass forms are recognized by the MHS-5 monoclonal on immunoblots in addition to the two major high molecular mass forms. Both HMM-SV and SVSA undergo changes in molecular weight in concurrence with the time course of coagulum liquefaction. SVSA shows several low molecular mass immunoreactive species, with a doublet of peptides being predominant at 10.9 and 12.5 kDa. HMM-SV similarly was reported to be cleaved into a number of basic proteins, the major

one of which has been recently purified (Lilja et al., 1984). This major cleavage product has a molecular mass of 12.8 kDa on SDS-PAGE, close to the triplet of peptides recognized by the MHS-5 monoclonal after 24 h of liquefaction. The 12.8-kDa protein derived from HMM-SV has been sequenced (Lilja and Jeppsson, 1985) and has a calculated molecular mass of 5733 Daltons based on its 52 amino acid composition. N-terminal amino acid sequence analysis of this peptide shows it to be identical to a basic protein purified by Seidah and coworkers (1984) and shown to have inhibin-like activity (Ramasharma et al., 1984). It is possible that the low molecular weight peptides recognized by the MHS-5 monoclonal are identical to the 12.8 kDa cleavage product of HMM-SV, in which case the MHS-5 monoclonal will provide a useful method for purification of the peptide with inhibin-like activity from human semen. We are currently purifying the low molecular mass peptides recognized by monoclonal antibody MHS-5 after 24 h of liquefaction by using immunoaffinity chromatography to sequence them and compare amino acid sequences with the published sequence for the 12.8 kDa form of HMM-SV.

An important observation obtained from this study relevant to the purification of the degraded peptides was the common fate of SVSA. Each vasectomized semen sample screened demonstrated that similar immunoreactive degradative products were produced in the 32- to 48-h liquefaction interval. These transformations in mass of SVSA over time following ejaculation will allow for the pooling of seminal fluid samples at similar liquefaction intervals for immunoaffinity purification and biochemical characterization of the degraded peptides.

Several enzymes of prostatic origin have been characterized and postulated to play a role in human semen liquefaction. These enzymes include the collagenase-like enzyme (Lukac and Koren, 1979), seminin (Tauber et al., 1980), and Suc$(Ala)_3$pNA hydrolyzing peptidase (Lilja and Laurell, 1984). Recently, prostate-specific antigen has been shown to be a serine protease (Watt et al., 1986). Purified prostate-specific antigen cleaves the seminal vesicle proteins shown by Lilja et al. (1985) to be a constituent of the coagulum. Studies to determine whether prostate-specific antigen and/or other uncharacterized seminal enzymes participate in the proteolysis of SVSA are in progress.

REFERENCES

Amelar RD, 1962. Coagulation, liquefaction and viscosity of human semen. J Urol 87:187–90

Edwards JJ, Tollaksen SL, Anderson NG, 1981. Proteins of human semen. 1. Two-dimensional mapping of human seminal fluid. Clin Chem 28(8):1335–40

Evans RJ, Herr JC, 1986. Immunohistochemical localization of the MHS-5 antigen in principal cells of human seminal vesicle epithelium. Anat Rec 214:372–77

Ey, PL, Prowse SJ, Jenkins CR, 1978. Isolation of pure IgG_1, IgG_{2a}, and IgG_{2b} immunoglobulins from mouse serum using protein A-sepharose. Immunochemistry 15:429–36

Herr JC, Fowler Jr, JE, Howards SS, Sigman M, Sutherland WM, Koons DJ, 1985. Human antisperm monoclonal antibodies constructed postvasectomy. Biol Reprod 32:695–711

Herr JC, Summers TA, McGee RS, Sutherland WM, Sigman M, Evans RJ, 1986. Characterization of a monoclonal antibody to a conserved epitope on human seminal vesicle-specific peptides: a novel probe/marker system for semen identification. Biol Reprod 35: 773–84

Koren K, Lukac J, 1979. Mechanism of liquefaction of the human ejaculate. 1. Changes of the ejaculate proteins. J Reprod Fertil 56: 493–99

Laemmli UK, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. Nature (London) 227:690–85

Leonardi D, Colpi GM, Campana A, Balerna M, 1983. Protein characterization of multi-fraction split ejaculates. Some physiochemical properties of prostatic and vesicular proteins. Acta Europaea Fertilitatis 14(3):181–89

Lilja H, 1985. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J Clin Invest 76:1899–903

Lilja H, Jeppsson JO, 1985. Amino acid sequence of the predominant basic protein in human seminal plasma. Febs Lets 182:181–84

Lilja H, Laurell CB, 1984. Liquefaction of coagulated human semen. Scand J Clin Lab Invest 44:447–52

Lilja H, Laurell CB, 1985. The predominant protein in human seminal coagulate. Scand J Clin Lab Invest 45:635–41

Lilja H, Laurell CB, Jeppsson JO, 1984. Characterization of the predominant basic protein in human seminal plasma, one cleavage product of the major seminal vesicle protein. Scand J Clin Lab Invest 44:439–46

Lilja H, Weiber H, 1984. Synthetic protease inhibitors and post-ejaculatory degradation of human semen proteins. Scand J Clin Lab Invest 44:433–38

Lukac J, Koren E, 1979. Mechanism of liquefaction of the human ejaculate. II. Role of collagenase-like peptidase and seminal proteinase. J Reprod Fertil 56:501–06

Notides AC, Williams-Ashman HG, 1967. The basic proteins responsible for clotting of guinea pig semen. Proc Nat Acad Sci 58:1991–95

Ramasharma K, Sairam MR, Seidah NG, Chetien M, Manjunath P, Schiller PW, Yamashiro D, Li CH, 1984. Isolation, structure, and synthesis of a human seminal plasma peptide with inhibin-like activity. Science 223:1199–202

Reynolds AB, Oliphant G, 1984. Production and characterization of monoclonal antibodies to the sperm acrosome stabilizing factor (ASF): utilization for purification and molecular analysis of ASF. Biol Reprod 30:775–86

Rui H, Mevag B, Purvis K, 1984. Two-dimensional electrophoresis of proteins in various fractions of the human split ejaculate. Int J Androl 7:509–20

Schon E, Lukac J, Milkovic S, 1984. A new proteinaceous factor participating in the rat ejaculate coagulation–identification, isolation and function. Biol Reprod 26:875–84

Seidah NG, Ramasharma K, Sairam MR, Chetien M, 1984. Partial amino acid sequence of a human plasma peptide with inhibin-like activity. Febs Let 167:98–102

Tauber PF, Propping D, Schumacher GFB, Zaneveld LJD, 1980a. Biochemical aspects of the coagulation and liquefaction of human

semen. J Androl 1:280–88
Tauber PF, Zaneveld LJD, Propping D, Schumacher GFB, 1976. Components of human split ejaculates. II. Enzymes and proteinase inhibitors. J Reprod Fertil 46:165–71
Tauber PF, Zaneveld LJD, Propping D, Schumacher GFB, 1980b. A new technique to measure the liquefaction rate of human semen: the bag method. Fertil Steril 33(5):567–70
Towbin H, Staehelin T, Gordon J, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–54
Watt KWK, Lee PJ, Timkulu TM, Chan WP, Loor R, 1986. Human prostate-specific antigen: structural and functional similarity with serine proteases. Proc Natl Acad Sci USA 83:3166–70
Williams-Ashman HG, 1984. Transglutaminases and the clotting of mammalian seminal fluids. Molec Cell Biochem 58:51–61