Sertoli Cell Synthesizes and Secretes a Protease Inhibitor, α_2-Macroglobulin†

C. Yan Cheng,*,‡,§ Josephine Grima,‡ Michael S. Stahler,‡ Angelo Guglielmotti,‡ Bruno Silvestrini,∥ and C. Wayne Bardin‡

The Population Council, 1230 York Avenue, New York, New York 10021, The Rockefeller University, 1230 York Avenue, New York, New York 10021, and Institute of Pharmacology and Pharmacognosy, University of Rome, Rome, Italy

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ABSTRACT: The mechanism by which the seminiferous epithelium limits the damaging effects of proteases that are released from degenerating late spermatids does not depend upon protease inhibitors in the systemic circulation since these proteins are excluded from the seminiferous tubule by the blood–testis barrier. The purpose of this study was to identify the major protease inhibitor of the testis and determine its cellular origin. Sertoli cells, the major epithelial component of the seminiferous epithelium, release a protease inhibitor, testicular α_2-macroglobulin, in vitro. Immunoprecipitation using [^{35}S]methionine and a monospecific polyclonal antibody prepared against purified testicular α_2-macroglobulin establishes that this protein is actively synthesized and secreted by Sertoli cells. Measurements of immunoreactive protease inhibitors in tubular and rete testis fluids collected by micropuncture suggest that α_2-macroglobulin rather than α_1-antitrypsin is the major protease inhibitor in the seminiferous tubules in vivo. The ability of α_2-macroglobulin to inactivate proteases and growth factors such as TGF-β by a common mechanism suggests that this protein may have a dual function in the testis.

In the testis, germ cells complete their development in the portion of the seminiferous epithelium that is behind the blood–testis barrier, remote from cells and proteins present in the systemic circulation. This barrier is formed by the junctional complexes between the basal portions of adjacent Sertoli cells (Fawcett, 1975; Dym, 1973), the major epithelial component of the seminiferous tubules. The portion of each seminiferous tubule that is behind the blood–testis barrier is termed the adluminal compartment, and this is continuous with the ductual system of the male reproductive tract (Fawcett, 1975; Dym, 1973), whereas the portion of each tubule outside the blood–testis barrier, the basal compartment, is contiguous with the interstitial space of the testis that contains the Leydig cells as well as the lymphatic and blood vessels. Since most of the macromolecules in blood and lymph cannot pass through the blood–testis barrier into the adluminal compartment, the microenvironment in which spermatogenesis and spermiogenesis occur must be created and maintained by cells of the seminiferous epithelium. A series of studies have now established that Sertoli cells are important regulators of this microenvironment (Bardin et al., 1988). To accomplish this, Sertoli cells synthesize and secrete transport proteins, growth factors, and attachment factors as well as proteins of unknown function into the adluminal compartment (Bardin et al., 1988; Cheng et al., 1988a; Cheng & Bardin, 1986, 1987; Griswold et al., 1986; Joseph et al., 1987; French & Ritzen, 1973; Wright et al., 1981). Thus, Sertoli cells supply many of the macromolecules for the adluminal compartment that hepatocytes provide for the systemic circulation.

Of all the proteins in the tubular lumen, no protease inhibitors have been identified other than the low molecular weight (M_r 6500) inhibitor associated with spermatozoa (Zaneveld et al., 1975). This is of physiological significance since the degeneration of thousands of late spermatids in the seminiferous tubule each day is associated with release of proteases, including the serine protease acrosin (EC 3.4.21.10), that could severely damage the seminiferous tubules and the rest of the genital tract if not inactivated (Polakoski & McRorie, 1973; Polakoski & Parrish, 1977). We reasoned, therefore, that protease inhibitors should be relatively abundant in the tubular fluid and that they would likely be secreted by Sertoli cells. Since Sertoli cells secrete many of the same proteins in vitro that they release in vivo (Bardin et al., 1988), protease inhibitors were sought in medium from Sertoli cell enriched cultures.

Materials and Methods

Biochemicals. ^{125}I-Labeled Bolton–Hunter [*N*-succinimidyl 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionate, specific activity 3024–3320 Ci/mmol] was obtained from ICN Radiochemicals (Irvine, CA). L-[^{35}S]Methionine (specific activity 1120–1224 Ci/mmol), *methyl*-^{14}C-methylated protein markers, and Enlightning autoradiography enhancer were from NEN (Boston, MA). Acrylamide, *N,N'*-methylenebis(acrylamide), immunoprecipitin (formalin-fixed *Staphylococcus aureus* cells), and prestained high molecular weight markers were from BRL (Gaithersburg, MD). Glycine, sodium dodecyl sulfate (SDS),[1] DATD (*N,N'*-diallyltartardiamide), TEMED (*N,N,N',N'*-tetramethylethylenediamine), and high and low molecular weight standards were from Bio-Rad (Richmond, CA). Protein A–peroxidase, 4-chloro-1-naphthol, and bovine serum albumin (fraction V, RIA grade) were from Sigma (St. Louis, MO).

Collection of Serum and Testicular Fluids. Blood was withdrawn by cardiac puncture from animals under light ether anesthesia, and sera were prepared as previously described (Cheng et al., 1984). Seminiferous tubular fluid was collected

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* Address correspondence to this author at The Population Council.
‡ The Population Council.
§ The Rockefeller University.
∥ University of Rome.

[1] Abbreviations: PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; TαMG, testicular α_2-macroglobulin; PTH-amino acids, phenylthiohydantoin-amino acids.

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by in vitro micropuncture (Howards et al., 1975; Turner et al., 1984). Rete testis fluid was collected by micropuncture 4 h after ligation of efferent ducts (Cheng et al., 1986a).

Preparation of Sertoli Cell Enriched Culture Medium. Primary Sertoli cell enriched cultures were prepared from testes of 20-day-old Sprague-Dawley rats as previously described (Cheng et al., 1986b; Mather & Sato, 1979). The procedures used in this laboratory to isolate Sertoli cells yielded preparations of greater than 95% pure which are basically freed of Leydig cells and germ cells contaminations when examined microscopically (Mather et al., 1982; Rich et al., 1983). Briefly, Sertoli cell aggregates were seeded at a density of approximately 5×10^6 cells per 9 mL of F12/DME (1:1 w/w) serum-free conditioned medium (Gibco) supplemented with insulin (10 μg/mL), transferrin (5 μg/mL), bacitracin (5 μg/mL), epidermal growth factor (2.5 ng/mL), testosterone (2×10^{-7} M), and FSH (300 ng/mL). The cells were maintained at 35 °C in a humidified culture chamber with 95% air and 5% CO_2. A batch of 5 L of Sertoli cell enriched culture medium was processed for isolation of testicular α_2-macroglobulin.

Protease Inhibition Assay. Protease inhibitor was assayed by using various proteases and methyl-α-[^{14}C]casein by established procedures (Quigley & Armstrong, 1983; Spycher et al., 1987). Protease inhibitor was identified by incubating proteases (0.05–0.1 μg of protein each) in the presence or absence of column fractions or purified TαMG (2–3 μg of protein) in 200 μL of protease buffer (0.025 M Tris, pH 7.4 at 22 °C, containing 3% NaCl; 10 mM $CaCl_2$ was included in the protease buffer for thermolysin; 1 mM EDTA and 5 mM cysteine were included in the protease buffer for papain) and [^{14}C]casein (10 μL, 0.5 μCi/mL) in an Eppendorf microcentrifuge tube. The reaction was allowed to proceed for 30 min at 22 °C. Thereafter, 200 μL of ice-cold trichloroacetic acid (20% w/v) and 300 μg of BSA were added to each tube to percipitate the unreacted [^{14}C]casein. Following sedimentation in an Eppendorf microcentrifuge for 2 min, hydrolyzed fragments in the supernatant were quantified by spectrometry using 5 mL of Liquiscint in a β-counter (Packard Model 3330) at 28% counting efficiency.

Purification of a Testicular Protease Inhibitor. (*A*) *Anion-Exchange HPLC.* A 5-L pool of primary Sertoli cell enriched culture medium was concentrated and equilibrated in solvent A (20 mM Tris, pH 7.4 at 22 °C) and prepared for anion-exchange HPLC using a Mono Q column (HR 10/16, 16 × 100 mm i.d.) using procedures as previously described (Cheng & Bardin, 1986, 1987).

(*B*) *Chromatofocusing HPLC.* Fractions containing protease inhibitor located by the assay described above were pooled and equilibrated in 0.025 M Bis-Tris, pH 7.1, adjusted with iminodiacetic acid at 22 °C and fractionated by chromatofocusing HPLC on a Mono P column (5 × 200 mm i.d.) as previously described (Cheng & Bardin, 1986). Bound proteins were eluted with Polybuffer 74:H_2O (1:10 v/v, pH 4.1 at 22 °C), and fractions containing protease inhibitor were pooled and concentrated to about 200 μL using an Amicon Centricon-10 microconcentrator.

(*C*) *Gel Permeation HPLC.* The concentrated sample was fractionated by gel permeation HPLC (Bio-Sil TSK-250, 7.5 × 300 mm i.d.) using 10 mM sodium phosphate/0.15 M NaCl, pH 6.8 at 22 °C, as an elution buffer as previously described (Cheng & Bardin, 1986, 1987).

NH_2-Terminal Sequence Analysis. Edman degradation was performed in an Applied Biosystem Inc. Model 470A gas-phase protein sequencer using native or reduced and S-carboxymethylated protein, and PTH-amino acids released from Edman degradations were identified by reverse-phase HPLC as previously described (Cheng et al., 1988a,b).

Immunoprecipitation of [^{35}S]Methionine-Labeled Testicular Protease Inhibitor. To determine if Sertoli cells actively synthesize and secrete protease inhibitors in vitro, cultures enriched for these cells were prepared from testes of 20-day-old rats and cultured in F12/DME medium containing one-tenth of the original concentration of methionine (Cheng et al., 1987). Each plate (9 mL each containing about 4.5×10^6 cells) was incubated with 100 μCi of [^{35}S]methionine for 24 h. Thereafter, a total of 17 mL of spent medium was collected and dialyzed extensively against 20 mM Tris, pH 7.4 at 22 °C, using a dialysis membrane with a molecular weight cutoff of 6000–8000 for 24 h at 4 °C to remove unbound [^{35}S]-methionine. Immunoprecipitation of [^{35}S]methionine-labeled proteins was performed as previously described (Cheng et al., 1987).

General Methods. Protein estimation by the dye binding assay using BSA as a standard was performed as previously described (Bradford, 1976). Antiserum was prepared in a New Zealand female rabbit using established procedures as previously described (Cheng & Bardin, 1986, 1987; Cheng et al., 1988b). The monospecificity of this antiserum was demonstrated by crossed-immunoelectrophoresis and immunoblots.

Results

Purification and NH_2-Terminal Sequence Analysis of a Testicular Protease Inhibitor. By use of an assay based on the hydrolysis of [^{14}C]casein by trypsin, an inhibitor was identified in media from Sertoli cell enriched cultures. This protein was purified to apparent homogeneity using sequential anion-exchange, chromatofocusing, and gel permeation HPLC (Figure 1A–C). Eight batches of this protein were isolated with an overall recovery of about 15%. The purified protein when resolved by SDS–PAGE under reducing conditions displayed a single band of M_r 175 000 (Figure 1C). Analysis of two separate batches of purified protein using an Applied Biosystem Inc. Model 470A gas-phase sequencer yielded a single NH_2-terminal sequence of NH_2-XAPQKPIYMVMVPSLLH which is identical with the NH_2-terminal sequence of rat serum α_2-macroglobulin (Gehring et al., 1987), except that the first NH_2-terminal amino acid of the testicular protein could not be conclusively assigned, which is a Ser in the serum protein. If the purified protease inhibitor was indeed α_2-macroglobulin, then it should inhibit a wide spectrum of proteases such as trypsin, papain, and thermolysin (Sottrup-Jensen, 1987). The results shown in Table I indicate that the protease inhibitor present in Sertoli cell enriched cultures has this biological activity of α_2-macroglobulin.

Subunit Structure Analysis of the Testicular Protease Inhibitor. Analysis of the purified protease inhibitor by electrophoresis revealed that it migrated as single bands of M_r 170 000 and 340 000 on SDS–polyacrylamide gels under reducing (Figure 2A) and nonreducing (Figure 2B) conditions, respectively. Thus, two monomers appear to be linked by disulfide bond(s). The molecular weight of the native protein as estimated by gel permeation HPLC using a Du Pont column (Zorbax GF-250, 9.4 × 250 mm i.d., equipped with a Bio-Sil TSK guard column, 7.5 × 75 mm i.d.) was 680 000, suggesting that two dimers are associated by noncovalent forces to form a tetramer (data now shown). Thus, the purified protease inhibitor isolated from Sertoli cell enriched cultures appears to be identical with serum α_2-macroglobulin which is also a tetramer composed of two pairs of disulfide-bonded monomers

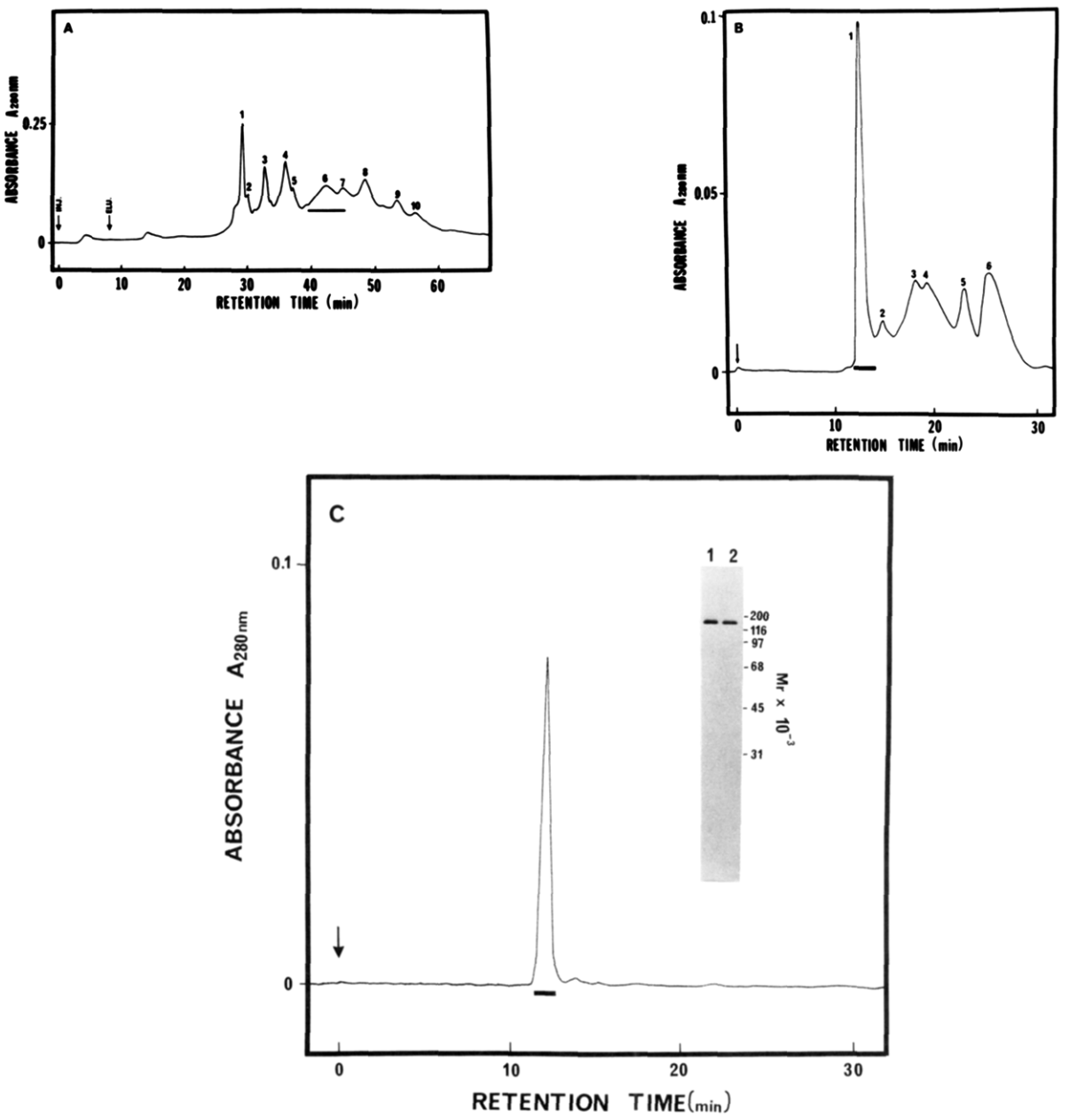

FIGURE 1: (A–C) Purification of a protease inhibitor from Sertoli cell enriched cultures. Sertoli cell enriched cultures were prepared from testes of 20-day-old Sprague-Dawley rats using established procedures (Cheng et al., 1986b; Mather & Sao, 1979). Five liters of medium containing about 200 mg of total protein was prepared for preparative anion-exchange HPLC using a Mono Q (HR 10/16, 16 × 100 mm i.d.) column as previously described (Cheng et al., 1989). Briefly, media were concentrated and equilibrated against solvent A (20 mM Tris, pH 7.4 at 22 °C) using an Amicon YM-10 membrane and were loaded onto this column at a flow rate of 4 mL/min. Bound proteins were eluted by using a linear salt gradient of 0–80% solvent B (20 mM Tris/600 mM NaCl, pH 7.4 at 22 °C) over a period of 45 min at a flow rate of 4 mL/min. Fractions containing the protease inhibitor as identified by the one-point protease assay described under Materials and Methods were pooled, concentrated, equilibrated against 0.025 M Bis-Tris, pH 7.1, adjusted with iminodiacetic acid at 22 °C, and loaded onto a Mono P chromatofocusing HPLC column (5 × 200 mm i.d.). The bound proteins were eluted with Polybuffer 74/H_2O (1:10 v/v, pH 4.1, adjusted with iminodiacetic acid at 22 °C). Of the 10 protein peaks visualized, the inhibitor was eluted between pH 4.2 and 5.1 under peaks 6–7 (A). These fractions were pooled, equilibrated against PBS buffer (10 mM sodium phosphate/0.15 M NaCl, pH 6.8 at 22 °C), and concentrated to about 200 μL by using an Amicon Centricon-10 microconcentrator. The sample was loaded onto a gel permeation HPLC column (Bio-Sil TSK-250, 7.5 × 300 mm i.d.) equipped with a guard column of 7.5 × 75 mm i.d. (Bio-Sil TSK guard column) at a flow rate of 0.5 mL/min. Proteins were eluted with PBS buffer; a total of six protein peaks were identified, and the protease inhibitor was eluted under peak 1 (B). Highly purified protease inhibitor obtained under peak 1 shown in (B) was concentrated to about 200 μL by using an Amicon Centricon-10 and fractionated on a separate Bio-Sil TSK-250 gel permeation HPLC column (300 × 7.5 mm i.d.). The purified protease inhibitor eluted as a single peak (C). When this protein was fractionated under reducing conditions on a SDS–polyacrylmaide gel (10% T), a single band was visualized with silver staining (Cheng et al., 1989) with an apparent molecular weight of 170 000. Lanes 1 and 2 shown in the insert of (C) represent column fractions under the protein peak shown in (C). Each lane consisted of about 0.3 μg of protein. The horizontal bar indicates fractions with protease inhibitor activity which was monitored with the protease inhibition assay using [^{14}C]casein and trypsin as described under Materials and Methods using a trypsin:column fraction ratio of 0.05 μg to 50–100 μL (containing about 2–4 μg of protein). The arrow at time zero indicates the sample injection.

(Gehring et al., 1987; Barrett et al., 1979; Jensen & Sottrup-Jensen, 1986; Sottrup-Jensen et al., 1984). In view of the biological and structural similarities between serum α_2-macroglobulin and the testicular protease inhibitor, we now propose that this latter protein be designated testicular α_2-macroglobulin (TαMG).

Secretion of TαMG by Sertoli Cell Enriched Cultures. A monospecific polyclonal antiserum was prepared against highly

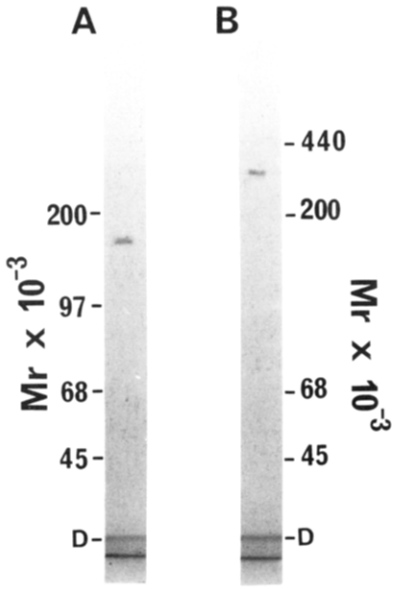

FIGURE 2: Subunit structure of testicular α_2-macroglobulin. The fractionation of the purified protease inhibitor on 6% T SDS–polyacrylamide gels under reducing (A) and nonreducing (B) conditions demonstrated proteins of apparent M_r 170 000 and 340 000, respectively. These results suggest that two monomers are linked by disulfide bonds. The molecular weight makers used for SDS–PAGE were the following: apoferritin, 443 000; myosin, 200 000; phosphorylase *b*, 97 000; BSA, 68 000; ovalbumin, 45 000.

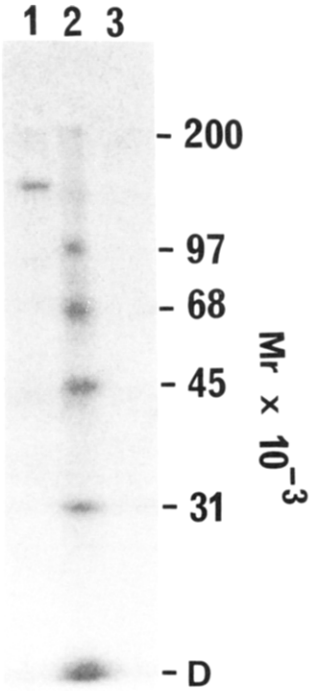

FIGURE 3: Sertoli cell secretes testicular α_2-macroglobulin in vitro. Sertoli cell enriched cultures (9 mL consisted of about 4.5×10^6 cells) were incubated with 10 μCl of [^{35}S]methionine for 12 h; thereafter, the [^{35}S]methionine-labeled proteins were immunoprecipitated using monospecific polyclonal antibodies prepared against purified TαMG. Immunoprecipitated proteins were fractionated by SDS–PAGE on a 10% T SDS–polyacrylamide gel under reducing conditions. Lane 1, [^{35}S]methionine-labeled proteins immunopreciptiated with anti-TαMG antibodies; lane 2, ^{14}C-labeled marker proteins; lane 3, radiolabeled proteins immunoprecipitated by using hyperimmune serum. Following electrophoresis, proteins in the gel were visualized by exposure to X-ray film (Kodak XAR-2) for 30 h at −70 °C.

purified TαMG for studies on the synthesis of this protein in vitro using Sertoli cell enriched cultures incubated with [^{35}S]methionine. When the [^{35}S]methionine-labeled proteins were reacted with anti-TαMG antibody, a single [^{35}S]-methionine-labeled protein that had a molecular weight identical with TαMG was immunoprecipitated (Figure 3), an observation consistent with the postulate that Sertoli cells synthesize and secrete this protein.

Table I: Inhibition of Hydrolysis of [^{14}C]Casein by Proteolytic Enzymes with TαMG[a]

enzyme (0.1 μg of protein)	TαMG (2 μg of protein)	hydrolysis products (cpm)
no enzyme	−	250 ● 15
	+	230 ± 25
trypsin	−	4880 ± 680
	+	2280 ± 150
	++ (4 μg of TαMG)	580 ● 89
papain	−	3920 ● 120
	+	1410 ± 170
thermolysin	−	4540 ± 140
	+	1890 ± 80

[a] Assays were performed as described under Materials and Methods using purified TαMG. Each number represents the result of triplicate determinations of a given experiment. These experiments were repeated twice using two separate batches of purified TαMG, and similar results were obtained in each experiment.

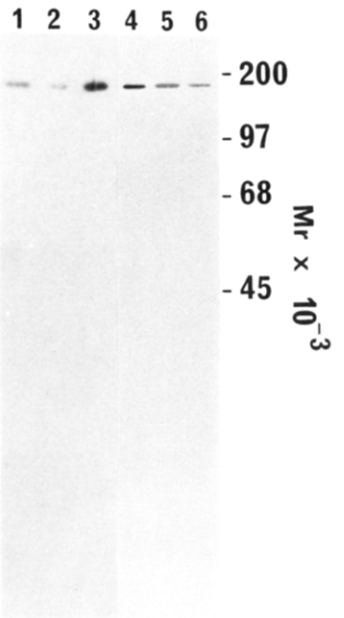

FIGURE 4: α_2-Macroglobulin in the reproductive tract fluids shares common epitopes with α_2-macroglobulin in rat and human serum. Normal rat serum (0.5 μL, 30 μg of protein, lane 1), normal human serum (0.5 μL, 30 μg of protein, lane 2), crude Sertoli cell enriched cultures (30 μL, 1 μg of protein, lane 3), highly purified TαMG (0.05 μg of protein, lane 4), rat rete testis fluid (0.2 μL, 0.2 μg of protein, lane 5), and seminiferous tubular fluid (0.1 μL, 2 μg of protein, lane 6) were resolved by SDS–PAGE on a 10% T SDS–polyacrylamide gel, transferred onto nitrocellulose paper, and immunologically stained using anti-TαMG antiserum.

Cross-Reactivity of Testicular α_2-Macroglobulin with Its Serum Homologue and Its Distribution in the Reproductive Tract. To determine whether the putative TαMG present in Sertoli cell enriched culture medium is immunologically related to serum α_2-macroglobulin, a highly sensitive radioimmunoassay was established. Both rat serum α_2-macroglobulin and TαMG exhibited parallel and complete competition with ^{125}I-TαMG for binding to TαMG antibodies (data not shown). The antibodies prepared against rat TαMG also cross-reacted with the human serum α_2-macroglobulin demonstrated by immunoblots (Figure 4). These observations suggest that the serum and testicular α_2-macroglobulins have similar epitopes.

To obtain evidence for the presence of TαMG in the adluminal compartment of the testis, seminiferous tubular fluid and rete testis fluid were collected by micropuncture. Immunoblots indicated that both fluids contain immunoreactive TαMG with the same molecular weight of the TαMG present in Sertoli cell enriched cultures (Figure 4). In rat rete testis fluid, immunoassayable TαMG constitutes about 10% of the total protein, and its concentration (70 ± 51 μg/mL, $n = 9$) is similar to that of α_2-macroglobulin in the serum (63 ± 30 μg/mL, $n = 4$). Analysis of immunoblots indicated that the concentration of TαMG in seminiferous tubular fluid is similar to that in rete testis fluid. The demonstration of immunoreactive TαMG in rete testis fluid and seminiferous tubular fluid coupled with the immunoprecipitation studies provides compelling evidence that this protease inhibitor is secreted behind the blood–testis barrier into the seminiferous tubular lumen by Sertoli cells.

In addition to α_2-macroglobulin, α_1-antitrypsin (M_r 54000) also inhibits a wide spectrum of proteases and is a predominant protease inhibitor in the systemic circulation (Schreiber, 1987). We, therefore, attempted to identify α_1-antitrypsin in Sertoli cell enriched culture medium by immunoblots using both polyclonal and monoclonal antibodies prepared against α_1-antitrypsin. In contrast to α_2-macroglobulin, immunoreactive α_1-antitrypsin was not detectable in the Sertoli cell enriched medium.

Discussion

α_2-Macroglobulin has been tentatively identified in ram rete testis fluid by immunoelectrophoresis using antisera prepared against crude ram serum and ram rete testis fluid (Johnson & Setchell, 1968); however, its molecular identity, subunit structure, and cellular origin in the testis have not been determined. The present study demonstrated that Sertoli cells are the source of the nonspecific protease inhibitor testicular α_2-macroglobulin in the testis. At present, we cannot rule out the possibility that other peritubular cells such as myoid cells and Leydig cells could synthesize and secrete TαMG and contribute to the testicular pool of TαMG. It was noted that TαMG has the same epitopes and subunit structure of the serum homologue. This protein was formally designated CMB-18 (Cheng et al., 1986b) and was first identified in primary Sertoli cell enriched cultures by HPLC and SDS–polyacrylamide gels.

α_2-Macroglobulin is an endopeptidase inhibitor in the blood of various mammals and the hemolymph of various invertebrates (Sottrup-Jensen, 1987; Quigley & Armstrong, 1983; Spycher et al., 1987; Saito & Sinohara, 1985). In the testis, this protein is believed to protect the seminiferous tubules and the rete testis from proteases released from spermatids and other cells. Its presence in the adluminal compartment of the seminiferous epithelium is primarily, if not entirely, dependent on the Sertoli cells. That Sertoli cells may not be unique in their ability to release this protein into a microenvironment is suggested by the synthesis and secretion of α_2-macroglobulin by fibroblasts, macrophages, and tumor cells (Mosher et al., 1977; Mosher & Wing, 1976; Bizik et al., 1986; Matoska et al., 1988).

α_2-Macroglobulin inhibits almost all known proteases examined to date irrespective of their catalytic mechanism(s) (Barrett & Starkey, 1973; Harpel, 1973). The proposed mechanism for its action involves cleavage of a susceptible peptide bond by the attacking protease, resulting in a conformational change of α_2-macroglobulin, which can then trap the protease (Barrett et al., 1979; Barrett & Starkey, 1973; Harpel, 1973). The entrapment process not only sterically hinders the enzyme but also results in formation of a covalent bond between the protease and an internal γ-glutamyl-β-cysteinyl thioester linkage of α_2-macroglobulin in the bait region (Sottrup-Jensen et al., 1980, 1987; Mortensen et al., 1981). Once a protease is bound to α_2-macroglobulin, the complex is cleared from the circulation by a receptor-mediated mechanism about 10 times faster than the unliganded inhibitor (Fuchs & Pizzo, 1983; Feldman & Pizzo, 1984). Receptors for α_2-macroglobulin have been demonstrated on fibroblasts and macrophages (van Leuven et al., 1986; Mosher & Vareri, 1980; Tycko & Maxfield, 1982; Kaplan & Nielsen, 1979). In the testis, the most likely site for such receptors would be the Sertoli cell since this is the major site of phagocytosis in the seminiferous epithelium.

In addition to its protease inhibitor activity, α_2-macroglobulin is also known to stimulate the growth of EC and RME cells in vitro (Feldman et al., 1984; Salomon et al., 1982). This activity may be explained in part by the findings that α_2-macroglobulin also binds a variety of growth factors including TGF-β (Huang et al., 1988; O'Connor-McCourt & Wakefield, 1987), which is also a product of Sertoli cells (Feig et al., 1983; Buch et al., 1988). The interaction of TGF-β with α_2-macroglobulin appears to have features in common with the protease–inhibitor interactions described above. That is, TGF-β is entrapped by and is covalently coupled to α_2-macroglobulin (Huang et al., 1988; O'Connor-McCourt & Wakefield, 1987). The majority of TGF-β in serum is bound to this protein so that it appears to be inactive in many bioassays. It has, therefore, been proposed that α_2-macroglobulin scavenges growth factors as well as proteases that are released at sites of injury.

On the basis of the assumption that the actions of α_2-macroglobulin in the testis are the same as in other organs, we propose a difunctional role for this protein. It is secreted into the adluminal compartment where it could limit the damage of proteases released by degenerated late spermatids and the seminiferous epithelium. An alternative functional role of the testicular α_2-macroglobulin is to inactivate proteases released by various testicular cells including myoid, Sertoli, and/or Leydig cells into the seminiferous tubular lumen. In addition, this protein could also maintain TGF-β in a latent form so that the specific actions of this factor could be exerted in only limited portions of the seminiferous epithelium.

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References

Bardin, C. W., Cheng, C. Y., Musto, N. A., & Gunsalus, G. L. (1988) in *The Physiology of Reproduction* (Knobil, E., & Neill, J., Eds.) pp 933–974, Raven Press, New York.

Barrett, A. J., & Starkey, P. M. (1973) *Biochem. J. 133*, 709–772.

Barrett, A. J., Brown, M. A., & Sayers, C. A. (1979) *Biochem. J. 181*, 401–418.

Bizik, J., Vaheri, A., Saksela, O., Kalkkinen, N., Meri, S., & Grofora, M. (1986) *Int. J. Cancer 37*, 81–88.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Buch, J. P., Lamb, D. J., Lipshultz, L. I., & Smith, R. G. (1988) *Fertil. Steril. 49*, 658–665.

Cheng, C. Y., & Bardin, C. W. (1986) *Biochemistry 25*, 5276–5288.

Cheng, C. Y., & Bardin, C. W. (1987) *J. Biol. Chem. 262*, 12768–12779.

Cheng, C. Y., Gunsalus, G. L., Musto, N. A., & Bardin, C. W. (1984) *Endocrinology 114*, 1386–1394.
Cheng, C. Y., Gunsalus, G. L., Morris, I. D., Turner, T. T., & Bardin, C. W. (1986a) *J. Androl. 7*, 175–179.
Cheng, C. Y., Mather, J. P., Byer, A. L., & Bardin, C. W. (1986b) *Endocrinology 118*, 480–488.
Cheng, C. Y., Grima, J., Lee, W. M., & Bardin, C. W. (1987) *Biol. Reprod. 37*, 875–885.
Cheng, C. Y., Chen, C. L. C., Feng, Z. M., Marshall, A., & Bardin, C. W. (1988a) *Biochem. Biophys. Res. Commun. 155*, 398–404.
Cheng, C. Y., Mathur, P. P., & Grima, J. (1988b) *Biochemistry, 27*, 4079–4088.
Cheng, C. Y., Grima, J., Stahler, M. S., Lockshin, R. A., & Bardin, C. W. (1989) *J. Biol. Chem. 264*, 21386–21393.
Dym, M. (1973) *Anat. Rec. 175*, 639–656.
Fawcett, D. W. (1975) in *Handbook of Physiology* (Hamilton, D. W., & Greep, O., Eds.) Vol. 5, pp 21–55, American Physiological Society, Washington, DC.
Feig, L. A., Klagsbrun, M., & Bellve, A. R. (1983) *J. Cell Biol. 97*, 1435–1443.
Feldman, S. R., & Pizzo, S. V. (1984) *Arch. Biochem. Biophys. 235*, 267–275.
Feldman, S. R., Gopnias, S. L., Ney, K. A., Pratt, C. W., & Pizzo, S. V. (1984) *J. Biol. Chem. 259*, 4458–4462.
French, F. S., & Ritzen, E. M. (1973) *Endocrinology 93*, 88–95.
Fuchs, H. E., & Pizzo, S. V. (1983) *J. Clin. Invest. 72*, 2041–2049.
Gehring, M. R., Shiels, B. R., Northemann, W., de Bruijn, M. H. L., Kan, C. C., Chain, A. C., Noonan, D. J., & Fey, G. H. (1987) *J. Biol. Chem. 262*, 446–454.
Griswold, M. D., Roberts, K., & Bishop, P. (1986) *Biochemistry 25*, 7265–7290.
Harpel, P. C. (1973) *J. Exp. Med. 138*, 508–521.
Howards, S. S., Johnson, A., & Jessee, S. (1975) *Fertil. Steril. 26*, 13–19.
Huang, S. S., O'Grady, P., & Huang, J. S. (1988) *J. Biol. Chem. 263*, 1535–1541.
Jensen, P. E. H., & Sottrup-Jensen, L. (1986) *J. Biol. Chem. 261*, 15863–15869.
Johnson, M. H., & Setchell, B. P. (1968) *J. Reprod. Fertil. 17*, 403–407.
Joseph, D. R., Hall, S. H., & French, F. S. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 339–343.
Kaplan, J., & Neilsen, M. L. (1979) *J. Biol. Chem. 254*, 7323–7328.
Mather, J. P., & Sato, G. H. (1979) *Exp. Cell Res. 120*, 191–200.
Mather, J. P., Perez-Infante, V., Zhuang, L. Z., & Phillips, D. M. (1982) *Ann. N.Y. Acad. Sci. 383*, 44–68.
Matoska, J., Wahlstrom, T., Vaheri, A., Bizik, J., & Grofora, M. (1988) *Int. J. Cancer 41*, 359–363.
Mortensen, S. B., Sottrup-Jensen, L., Hansen, H. F., Petersen, T. E., & Magnusson, S. (1981) *FEBS Lett. 135*, 295–300.
Mosher, D. F., & Wing, D. A. (1976) *J. Exp. Med. 143*, 462–467.
Mosher, D. F., & Vaheri, A. (1980) *Biochim. Biophys. Acta 627*, 113–122.
Mosher, D. F., Saksela, O., & Vaheri, A. (1977) *J. Clin. Invest. 60*, 1036–1045.
O'Connor-McCourt, M. D., & Wakefield, L. M. (1987) *J. Biol. Chem. 262*, 14090–14099.
Polakoski, K. L., & McRorie, R. A. (1973) *J. Biol. Chem. 248*, 8183–8188.
Polakoski, K. L., & Parrish, R. F. (1977) *J. Biol. Chem. 252*, 1888–1894.
Quigley, J. P., & Armstrong, P. B. (1983) *J. Biol. Chem. 258*, 7903–7906.
Rich, K. A., Bardin, C. W., Gunsalus, G. L., & Mather, J. P. (1983) *Endocrinology 113*, 2284–2293.
Saito, A., & Sinohara, H. (1985) *J. Biol. Chem. 260*, 775–781.
Salomon, A., Bano, M., Smith, K. B., & Kidwell, W. R. (1982) *J. Biol. Chem. 257*, 14093–14101.
Sottrup-Jensen, L. (1987) in *The Plasma Proteins* (Putnam, F. W., Ed.) Vol. 5, pp 191–291, Academic Press, New York.
Sottrup-Jensen, L., Petersen, T. E., & Magnusson, S. (1980) *FEBS Lett. 121*, 275–279.
Sottrup-Jensen, L., Stepanik, T. M., Kristensen, T., Wierzbicki, D. M., Jones, C. M., Lonblad, P. B., Magnusson, S., & Petersen, T. E. (1984) *J. Biol. Chem. 259*, 8318–8327.
Spycher, S. E., Arya, S., Isenman, D. E., & Painter, R. H. (1987) *J. Biol. Chem. 262*, 14606–14611.
Turner, T. T., Jones, C. E., Howards, S. S., Ewing, L. L., Zegeye, B., & Gunsalus, G. L. (1984) *Endocrinology 115*, 1925–1932.
Tycko, B., & Maxfield, F. R. (1982) *Cell 28*, 643–651.
van Leuven, T., Marynen, P., Sottrup-Jensen, L., Cassiman, J. J., & van den Berghe, H. (1986) *J. Biol. Chem. 261*, 11369–11373.
Wright, W. W., Musto, N. A., Mather, J. P., & Bardin, C. W. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 7565–7569.
Zaneveld, L. J. D., Polakoski, K. L., & Schumacher, G. F. B. (1975) in *Proteases and Biological Control: Conferences on Cell Proliferation* (Reich, R. E., Rifkin, D. B., & Shaw, E., Eds.) Vol. 2, pp 683–705, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.