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Seminalplasmin, the major basic protein of bull seminal plasma, is a secretory protein of the seminal vesicles

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From the experimental results of three independent methods: (1) indirect immunofluorescence employing monospecific anti-seminalplasmin-IgGs, (2) cell-free translation of poly(A)$^+$ RNA from seminal vesicle and testicular tissue, as well as (3) Northern analysis of poly(A)$^+$ RNA of the latter tissues with a synthetic seminalplasmin-specific antisense DNA probe, it is concluded that the biosynthesis of seminalplasmin occurs in seminal vesicles but not in testis.

Introduction

Bull seminal plasma contains a highly basic protein of 47 amino acids of known sequence [1–3]. This protein, originally discovered by Reddy and Bhargava [4] and named seminalplasmin (SAP), has been shown to possess a number of interesting biological properties: (1) antimicrobial activity [4,5], (2) calmodulin-antagonist function [6], (3) inhibition of uptake of Ca^{2+}-ions by epididymal bovine spermatozoa [7] and (4) inhibition of lymphocyte proliferation [8].

Although SAP could be purified from the secretion of bull seminal vesicles [9], an attempt to demonstrate the biosynthesis of SAP in this gland by immunohistochemical methods failed [10]. The reason for this failure might have been the use of an anti-SAP antiserum of low titer and specificity. We recently reported the characterisation and application of monospecific anti-SAP-IgGs [11]. In the following, we provide evidence by three independent methods: (1) immunohistochemistry, (2) cell-free translation of poly(A)$^+$ RNA as well as (3) Northern analysis of poly(A)$^+$ RNA for the biosynthesis of SAP in the seminal vesicles.

Abbreviations: SAP, seminalplasmin; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis.

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Materials and Methods

The preparation of poly(A)$^+$ RNA from seminal vesicle tissue and testis followed the protocol reported by Kemme et al. [12]. The preparation of mono-specific anti-SAP-IgGs is described elsewhere [11]. Rabbit reticulocyte lysate cell-free translation kit was from New England Nuclear (Dreieich). Dog pancreatic microsomal membrane preparation for protein processing as well as [^{35}S]methionine (spec.act. 400 Ci/mmol) were commercial products (Amersham, Braunschweig). The cell-free translation followed a protocol provided by the manufacturer of the kit. The standard reaction mixture (25 μl) contained 0.8 μg of poly(A)$^+$ RNA from seminal vesicle tissue or testis, respectively. Immunoabsorption was carried out with 20 μl of the cell-free translation standard reaction mixture employing 2 μg monospecific anti-SAP-IgGs and protein G-Sepharose according to Clemens [13]. An aliquot (25%) of the radioactive proteins purified by immunoabsorption was separated by SDS-PAGE under denaturing conditions according to Laemmli [14] using a 15% gel. The total mixture of radioactive proteins obtained in cell-free translation (0.5 μl of the standard reaction mixture) was separated by SDS-PAGE for comparison. Fluorography of the gel was performed with En^{3}Hance (New England Nuclear) following the manufacturers instructions. Synthetic DNA was synthesized using a model 381A DNA synthesizer (Applied Biosystems). The immunohistochemical experiments followed standard procedures. In short, kryosections of seminal vesicle tissue (5 μm) were

dried onto glass slides, fixed with formaldehyde (3.7%, w/v) for 10 min and washed with phosphate-buffered saline (PBS). Incubation with the primary antibody was carried out a concentration of 10 μg/ml in PBS; unspecific binding was suppressed by addition of 1% BSA in PBS. Detection of the primary antibody was achieved with rhodamin-labelled anti-rabbit-IgGs.

For Northern-transfer analysis, RNA after treatment by glyoxal and DMSO was electrophoresed in an 0.8% agarose gel [15] and transferred to GeneScreene membranes (New England Nuclear). For the hybridisation of RNA blots with DNA probes, we strictly followed the procedure of Khandjian [16]. DNA probes were ^{32}P-labelled by T4 polynucleotide kinase to a specific activity of 10^7 cpm/μg.

Results and Discussion

Formaldehyde-fixed kryosections of seminal vesicle tissue were incubated with monospecific rabbit anti-SAP-IgGs followed by incubation with rhodamin-conjugated anti-rabbit-IgGs. As shown in Fig. 1 positive immunoreactivity was found in secretory cells of the glandular epithelium; no reactivity was observed with preserum as control (data not shown). Sections from testis likewise displayed no reactivity against anti-SAP-IgGs (data not shown). The detection of the major protein of seminal plasma of bull, a secretory protein of seminal vesicles [17] by anti-major protein antiserum was used as a positive control (Fig. 1).

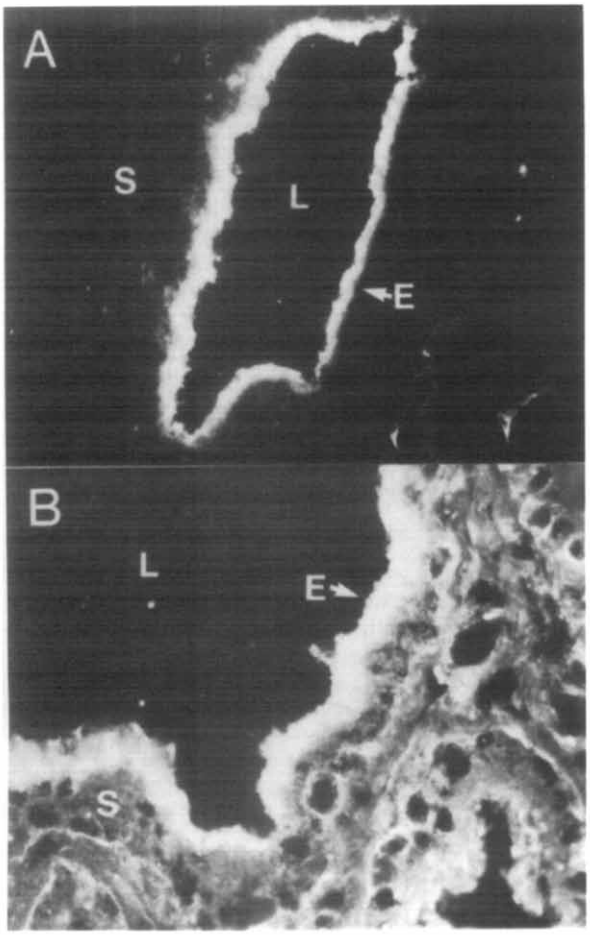

Fig. 1. Immunoreaction of seminal vesicle epithelium with monospecific antibodies against SAP (A, ×160) and major protein SVSP109 (B, ×400). Epithelium, E; stroma, S; lumen, L.

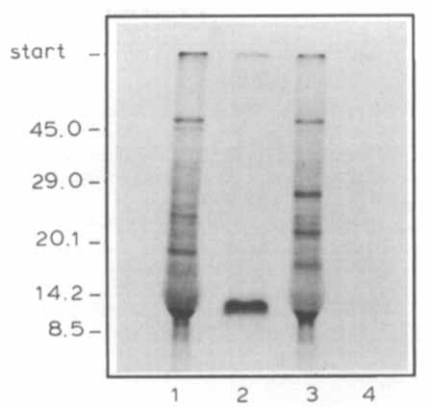

Fig. 2. Cell-free translation of poly(A)$^+$ RNA from seminal vesicle and testicular tissue of bull. (1) Poly(A)$^+$ RNA from seminal vesicle tissue; total mixture of [^{35}S]methionine-labelled proteins; (2) poly(A)$^+$ RNA from testicular tissue; total mixture of [^{35}S]methionine-labelled proteins; (4) poly(A)$^+$ RNA from testicular tissue; immunoabsorption with anti-SAP IgGs. For further details see Material and Methods. Values are molecular masses in kDa.

Poly(A)$^+$ RNA was prepared from total RNA of seminal vesicle as well as testicular tissue. Proteins synthesized in vitro under direction of mRNA were labelled with [^{35}S]methionine. When radioactive proteins were subjected to immunoabsorption using monospecific anti-SAP-IgGs and protein G-Sepharose, autoradiography of the isolated proteins separated by SDS-PAGE, revealed the presence of only one polypeptide with an apparent molecular mass of 10 kDa (Fig. 2). The fact that this protein is labelled by [^{35}S]methionine, whereas SAP itself does not possess methionine residues, indicates that the immunoabsorbed polypeptide represents the SAP-precursor. The higher molecular mass of 10 kDa compared to 6 kDa lends further support to this interpretation. Processing of this SAP precursor by a microsomal preparation from dog pancreas leads to disappearance of the precursor protein because the only [^{35}S]methionine residues are located in the signal peptide of the precursor (data not shown). Cell-free translation of poly(A)$^+$ RNA from testicular

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3´ CTG CTT TTC CGG AGG GGG CTG TTT GTG GTG GCC AAG 5´
       C   A   A   A   A       C   A   A   G   A

   D   E   K   A   S   P   D   K   H   H   R   F
   2           5                   10          13
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Fig. 3. SAP-specific DNA hybridisation probe, antisense DNA sequence corresponding to amino acid residues 2–13 of SAP.

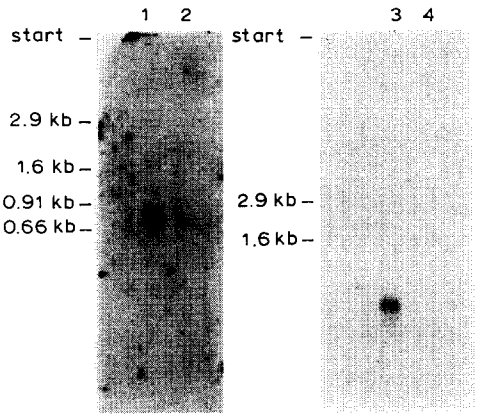

Fig. 4. Northern analysis of RNA from seminal vesicle and testicular tissue of bull (1) poly(A)$^+$ RNA (3 μg) from seminal vesicle; (2) total RNA (10 μg) from seminal vesicle; (3) poly(A)$^+$ RNA (2 μg) from seminal vesicle; (4) poly(A)$^+$ RNA (3 μg) from testicular tissue. In all hybridisations the specific activity of the probe was $4.6 \cdot 10^8$ cpm/μg.

tissue did not furnish any protein species immunoreactive with anti-SAP-IgGs (Fig. 2).

Poly(A)$^+$ RNA from seminal vesicle and testicular tissue as well as total RNA from seminal vesicle tissue were separated on an agarose gel and subjected to Northern analysis employing a synthetic DNA hybridisation probe. The latter was designed as antisense DNA for amino acid residues 2–13 of the SAP sequence, taking into consideration the codon usage tabulated from the GenBank genetic sequence data [18] as well as the cDNA sequences for the seminal RNAase BS1 [19] and the major protein SVSP94 [20] of bull semen (Fig. 3). Northern analysis under the conditions of high-stringency hybridisation indicated the presence of one SAP-specific mRNA species of 750 bp (Fig. 4). With poly(A)$^+$ RNA from testis a negative result was obtained, which is in agreement with the immunohistochemical data. A weak signal for SAP-specific mRNA could also be obtained when total RNA of seminal vesicle tissue was employed in Northern analysis. The lack of any hybridisation with rRNA in the latter experiment indicates the specificity of the hybridisation conditions employed.

From the experimental results using three independent methods: (1) immunochistochemistry, (2) cell-free translation of poly(A)$^+$ RNA as well as (3) Northern analysis with a synthetic DNA hybridisation probe, we conclude that the biosynthesis of SAP occurs in the accessory sex gland seminal vesicle.

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