Seminalplasmin and caltrin are the same protein

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The sequence of seminalplasmin, a basic antimicrobial and transcription-inhibitory protein from bovine seminal plasma, has been determined using an automated sequenator. This sequence is slightly different from that reported earlier by Theil and Scheit [(1983) EMBO J. 2, 1159–1163] and identical with that of caltrin, a Ca^{2+}-transport-inhibitory protein of bovine seminal plasma. Caltrin and seminalplasmin are, therefore, the same protein.

Seminalplasmin *Caltrin* *Seminal plasma protein*

1. INTRODUCTION

Seminalplasmin is an antimicrobial, transcription-inhibitory protein isolated from bovine seminal plasma several years ago in our laboratory [1–4]. It appears to act on a wide spectrum of bacteria and yeast by entering the cell and inhibiting RNA synthesis [5–7]. It has been shown to bind strongly to RNA polymerases and reverse transcriptases [8,9]. It also appears to act as an antagonist of calmodulin [10].

In 1983, Theil and Scheit [11] published the sequence of seminalplasmin, indicating it to be a small peptide of 48 amino acids. The sequence was determined by an analysis of the tryptic and chymotryptic fragments of seminalplasmin. Recently, Lewis et al. [12] obtained the sequence, using an automated sequenator, of a 47 amino acid protein which they called 'caltrin' and which was also isolated from bovine seminal plasma; caltrin inhibits the transport of Ca^{2+} in bovine spermatozoa [12]. This sequence was identical with that reported by Theil and Scheit [11] for seminalplasmin in respect of the first 24 amino acids; however, the sequence of caltrin had two segments, residues 24–34 and 35–45, in reversed order from that reported for seminalplasmin by Theil and Scheit. Lewis et al. also did not detect the lysine residue reported by Theil and Scheit in their position 46, making caltrin one amino acid shorter than seminalplasmin if the sequence of Theil and Scheit were to be correct. Lewis et al. suggested that caltrin may be identical to seminalplasmin and that the sequence reported by Theil and Scheit could be in error in regard to the reversed order of the two segments mentioned and the extra lysine in position 46.

We have now sequenced, purified RNase-free seminalplasmin in a single run on an automated sequenator, and report that the sequence of seminalplasmin is identical to that of caltrin. Thus, caltrin and seminalplasmin are the same protein.

2. MATERIALS AND METHODS

2.1. *Materials*

Bovine seminal plasma was purchased from Bharatiya Agro-Industries Foundation, Poona; HPLC-grade methanol, acetonitrile and *n*-butanol from Spectrochem, Bombay; and phenylthiohydantoin (PTH)-amino acid standards and trifluoroacetic acid (TFA, Sequenal grade) from Pierce. Water was purified by passing quartz double-distilled water through a Bondapak C18/Porasil B column (Waters).

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Seminalplasmin was isolated as described by Reddy and Bhargava [1] except that the final purification after the Sephadex G-75 run was carried out by reverse-phase liquid chromatography on a Waters μ-Bondapak C_{18} column (3.9 × 300 mm) using a gradient of 0–60% acetonitrile containing 0.1% TFA, in aqueous 0.1% TFA, in 60 min at a flow rate of 1 ml/min using curve no.5 (fig.1). The peak eluting at 35 min was seminalplasmin as determined by assaying for antibacterial activity. The high-performance liquid chromatography (HPLC) system (Waters) used consisted of two M-45 solvent delivery systems, a model 720 system controller, data module 710B WISP, a fixed-wavelength 440 absorbance detector at 254 nm, and a model 450 variable-wavelength detector.

2.2. *Sequencing*

Seminalplasmin (15 nmol) from the HPLC run was lyophilised, dissolved in water and directly applied on a glass-fiber filter containing biobrene precycled for 5 cycles. Sequencing was done by using a modified NVAC programme, on a 470 A gas-phase sequencer from Applied Biosystems.

2.3. *Identification of PTH-amino acids*

PTH derivatives were identified either by HPLC or by fast protein-liquid chromatography (FPLC) using a Waters μ-Bondapak C_{18} (3.9 × 300 mm) or a Pharmacia PepRPC (HR 5/5) column, respectively. For the HPLC separation (fig.2), the buffer system consisted of (A) 10 mM phosphate buffer (pH 6.6) containing 20% methanol and 0.5% *n*-butanol, and (B) methanol. A linear gradient of 0–65% solvent B in solvent A, in 35 min at a flow rate of 1.3 ml/min at 25°C, was used. For the separation on FPLC (fig.3), the buffer system consisted of (A) 6.6 mM phosphate buffer (pH 6.6) containing 13% methanol and 0.33% *n*-butanol, and (B) methanol. The gradient was 0% B from 0 to 5 min, 0–45% (linear gradient) B in A from 5–25 min, and 45–0% B in A from 25 to 28 min at a flow rate of 1 ml/min at 25°C. In both systems, all the PTH-amino acids except Val and Met, and Ile and Phe, were resolved; the Val-Met ambiguity did not matter in this case as seminalplasmin has no methionine [1].

3. RESULTS AND DISCUSSION

In the original method of isolation of seminalplasmin described by Reddy and Bhargava [1], dialysed seminal plasma was run sequentially on DEAE-Sephadex, CM-Sephadex, Sephadex G-75 and RNase affinity columns. The latter column was, however, often unable to remove the last traces of contaminating RNase activity from seminalplasmin. Theil and Scheit subsequently used an HPLC run on a Nucleosil C-18 column, using phosphate buffer, to separate seminalplasmin from the contaminating nuclease [13]. We have also used reverse-phase liquid chromatography on an HPLC system for obtaining homogeneous seminalplasmin, starting from the Sephadex G-75 eluted crude seminalplasmin [1]. However, we used TFA instead of phosphate buffer which allows direct evaporation to remove solvents. The results with this column are reproducible, and the seminalplasmin obtained is fully active [1]. A typical run on the HPLC system is given in fig.1.

We used seminalplasmin prepared as above on the HPLC system for sequencing, using an automated sequenator and either an HPLC or an FPLC system to identify the derivatised (PTH) amino acids. The FPLC and HPLC systems we used have not been used so far to separate PTH-amino acids. Typical runs of standard PTH-amino acids (except cysteine) on the HPLC and FPLC systems are given in figs 2 and 3, respectively; Ile and Phe, as well as Val and Met, were not separated. As seminalplasmin has no Met and Cys, the only ambiguity that could not be resolved was in regard to Phe and Ile; seminalplasmin has two Phe and one Ile residue per molecule.

Fig.4 gives the sequence of seminalplasmin determined by us during a single run on an automated amino acid sequenator. The sequences of caltrin as determined by Lewis et al. [12] and of seminalplasmin as determined by Theil and Scheit [11] are also given for comparison. Keeping in mind that our separation system could not resolve Ile and Phe, it would seem that the sequence of seminalplasmin is identical with that of caltrin, and differs from that of Theil and Scheit in regard to the order of the two segments, residues 25–34 and 35–45, and the presence of one lysine residue in position 46 of Theil and Scheit's sequence. Seminalplasmin and caltrin are, therefore, the

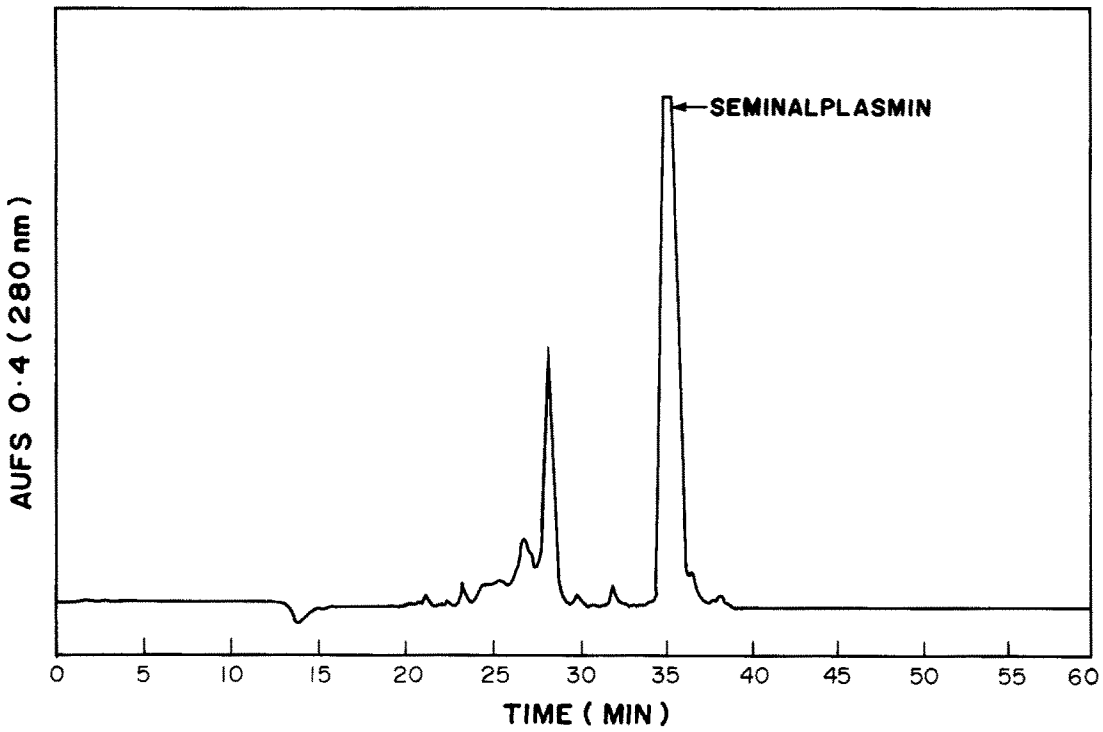

Fig.1. Purification of seminalplasmin by reverse-phase HPLC.

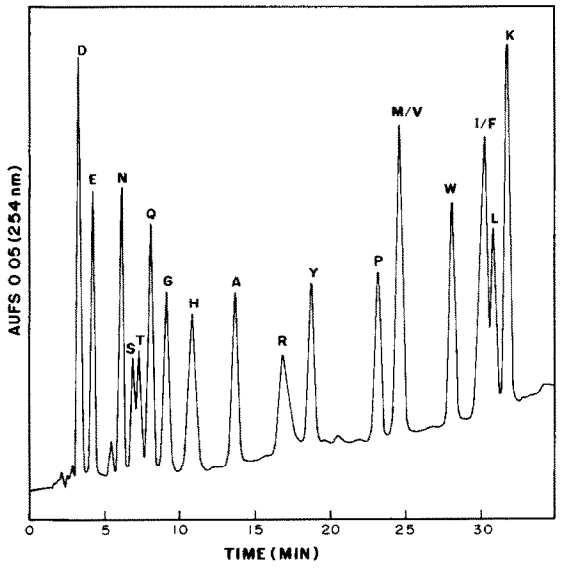

Fig.2. Separation of PTH-amino acids (500 pmol each) on a μ-Bondapak C_{18} column.

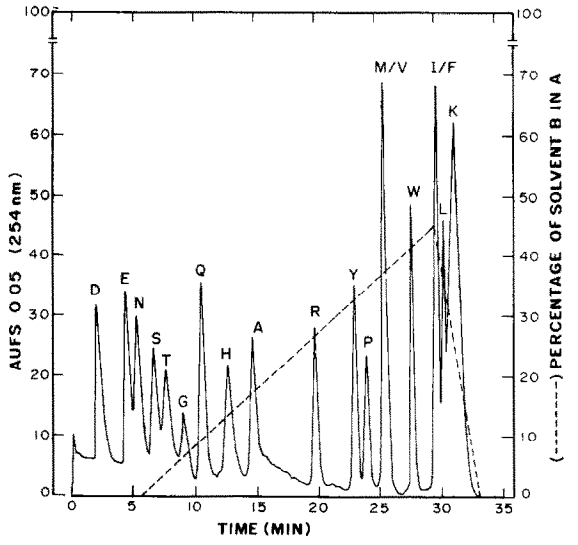

Fig.3. Separation of PTH-amino acids (400 pmol each) on a PepRPC column.

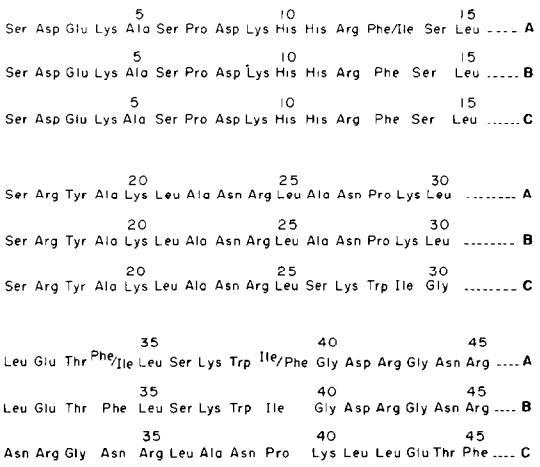

Fig.4. Amino acid sequence of seminalplasmin as determined in the present study (A), compared with the sequence of caltrin (B [12]) and of seminalplasmin as reported by Theil and Scheit (C [13]). In the present study, Ile and Phe could not be resolved.

same protein. We suggest that this protein continue to be called 'seminalplasmin' as this was the name first given to it [1].

Lewis et al. [12] have shown that caltrin inhibits transport of Ca^{2+} in epididymal spermatozoa. As Ca^{2+} plays an important role in fertilization [14,15], we are now investigating the role of seminalplasmin in the regulation and control of fertilization.

REFERENCES

[1] Reddy, E.S.P. and Bhargava, P.M. (1979) Nature 279, 725–728.
[2] Bhargava, P.M. (1981) Biochem. Soc. Trans. 9, 540–543.
[3] Bhargava, P.M. (1981) Proc. Natl. Acad. Sci. India, Annual Number, 51–57.
[4] Shivaji, S. (1984) Trend Biochem. Sci. 9, 104–109.
[5] Scheit, K.H., Zimmer, M., Rao, N.S., Prasad, K.S.N. and Bhargava, P.M. (1986) Microbios, in press.
[6] Scheit, K.H. and Bhargava, P.M. (1985) Ind. J. Biochem. Biophys. 22, 1–7.
[7] Scheit, K.H., Shivaji, S. and Bhargava, P.M. (1985) Jap. J. Biochem. 97, 463–471.
[8] Scheit, K.H., Reddy, E.S.P. and Bhargava, P.M. (1979) Nature 279, 728–731.
[9] Reddy, E.S.P., Das, M.R., Reddy, E.P. and Bhargava, P.M. (1983) Biochem. J. 209, 183–188.
[10] Giertzen, K. and Galla, H.J. (1985) Biochem. J. 230, 277–280.
[11] Theil, R. and Scheit, K.H. (1983) EMBO J. 2, 1159–1163.
[12] Lewis, R.V., Agustin, J.S., Kruggel, W. and Lardy, H.A. (1985) Proc. Natl. Acad. Sci. USA 82, 6490–6491.
[13] Theil, R. and Scheit, K.H. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1003–1009.
[14] Yanagimachi, R. and Usui, N. (1974) Exp. Cell Res. 89, 161–174.
[15] Edwards, R.G. (1985) Proc. Roy. Soc. Lond. B 223, 417–448.