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Cysteine-rich secretory proteins in snake venoms form high affinity complexes with human and porcine β-microseminoproteins

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ABSTRACT

β-Microseminoprotein (MSP), a 10 kDa protein in human seminal plasma, binds human cysteine-rich secretory protein-3 (CRISP-3) with high affinity. CRISP-3 is a member of the family of CRISPs, which are widespread among animals. In this work we show that human as well as porcine MSP binds catrin, latisemin, pseudecin, and triflin, which are CRISPs present in the venoms of the snakes *Crotalus atrox*, *Laticauda semifasciata*, *Pseudechis porphyriacus*, and *Trimeresurus flavoviridis*, respectively. The CRISPs were purified from the venoms by affinity chromatography on a human MSP column and their identities were settled by gel electrophoresis and mass spectrometry. Their interactions with human and porcine MSPs were studied with size exclusion chromatography and surface plasmon resonance measurements. The binding affinities at 25 °C were between 10^{-10} M and 10^{-7} M for most of the interactions, with higher affinities for the interactions with porcine MSP compared to human MSP and with Elapidae CRISPs compared to Viperidae CRISPs. The high affinities of the bindings in spite of the differences in amino acid sequence between the MSPs as well as between the CRISPs indicate that the binding is tolerant to amino acid sequence variation and raise the question how universal this cross-species reaction between MSPs and CRISPs is.

1. Introduction

β-Microseminoprotein (MSP) is a 10 kDa disulfide rich protein present in high concentration in human seminal plasma (Lilja and Abrahamsson, 1988). MSPs have been described in many vertebrates and the amino acid sequence is highly variable (Lazure et al., 2001; Wang et al., 2005b). Human MSP (hMSP), which has also been referred to as PSP94, is produced in the prostate gland (Doctor et al., 1986) and has also been identified in several other tissues including tracheal and gastric mucosa (Weiber et al., 1990). Being a major secretory product of the prostate gland, MSP has been studied especially with respect to prostate cancer (see Reeves et al., 2006) and the interest was intensified recently, when two genome-wide association studies reported that a locus on chromosome 10, which includes the gene for MSP, is associated with susceptibility to prostate cancer (Eeles et al., 2008; Thomas et al., 2008).

A number of functions of MSP have been suggested but none supported by convincing experimental evidence (Lazure et al., 2001). A possible clue to the function was the discovery that hMSP binds human cysteine-rich secretory protein-3 (CRISP-3) (Udby et al., 2005).

CRISP-3 belongs to a family of proteins described in mammals, snakes, lizards (see Roberts et al., 2007) and

Abbreviations: CRISP, cysteine-rich secretory protein; hMSP, human MSP; MS, mass spectrometry; MSP, β-microseminoprotein; nanoESI, nanoelectrospray ionization; pMSP, porcine MSP; PSP94, prostate secretory protein of 94 amino acids; PSPBP, PSP94-binding protein; RU, response unit; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SGP28, specific granule protein of 28 kDa.

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lamprey (Ito et al., 2007). They are distinguished by amino acid sequence characteristics such as molecular sizes of 22–25 kDa (200–250 amino acids), a high degree of amino acid sequence similarity, and a highly conserved specific pattern of 16 cysteines (Roberts et al., 2007).

In mammals four different CRISPs have been identified (see Roberts et al., 2007) and a recent study in the mouse has shown that the CRISPs are widely expressed in the organism (Reddy et al., 2008). In the human there are three CRISPs (Kratzschmar et al., 1996). Of these, CRISP-1 (acidic epididymal glycoprotein or AEG) and CRISP-2 (testis specific protein 1 or TPX-1) are expressed predominantly in the male reproductive tract and many studies indicate a function in sperm maturation and the fertilization process (Roberts et al., 2007; Gibbs et al., 2007). CRISP-3 was first demonstrated as an androgen-dependent transcript in the mouse salivary gland (Mizuki and Kasahara, 1992; Haendler et al., 1993) and its eight-exon gene was later characterized (Schwidetzky et al., 1995). The human CRISP-3 protein was first isolated from neutrophilic granulocytes, then called SGP28 (Kjeldsen et al., 1996) and later further characterized by Udby et al. (2002b). CRISP-3 is released from activated granulocytes (Udby et al., 2002a) and it is also expressed in other places, e.g. in the salivary gland, pancreas and prostate (Kratzschmar et al., 1996; Udby et al., 2002b).

The function of human CRISP-3 is unknown but several CRISPs have been shown to be ion-channel inhibitors. This has been shown for CRISP-2 from the mouse (Gibbs et al., 2006) and also for many CRISPs present in snake venoms: pseudechetoxin (Brown et al., 1999), pseudecin (Yamazaki et al., 2002a), ablomin, latisemin, triflin (Yamazaki et al., 2002b) and natrin (Wang et al., 2005a, 2006). Supposing that the putative function of human CRISP-3 is mediated via ion-channel inhibiting effects and considering the fact that human CRISP-3 is not only bound with high affinity by MSP in seminal plasma (Udby et al., 2005) but also by α1B-glycoprotein in blood plasma (Udby et al., 2004) it is natural to think that MSP and α1B-glycoprotein are important components of a system for protection against injurious systemic effects of CRISPs. MSP–CRISP interactions are not limited to the human. Recently, a new MSP (named SSP-2) was isolated from the blood of *Trimeresurus flavoviridis* and demonstrated to bind triflin, i.e. the CRISP present in the snakes' own venom (Aoki et al., 2007). SSP-2 has also been shown to form a complex with serotriflin, a new CRISP found in the blood of *T. flavoviridis* (Aoki et al., 2008).

Whether MSP binds human CRISP-1 and -2 is not known; that knowledge must await the isolation or recombinant production of these proteins. CRISPs from snake venoms are more easily available; several have been isolated and characterized (see Yamazaki and Morita, 2004).

Up to now, interaction between an MSP and a CRISP has only been observed in two species, i.e. in the human and the snake *T. flavoviridis*. These interactions are both intra-species, i.e. they take place between MSPs and CRISPs from the same species. An unpublished observation that porcine MSP also could bind human CRISP-3 raised the question how general the reaction is between MSPs and CRISPs, and if mammal MSPs could bind CRISPs from species as divergent as reptiles. In this work we show that hMSP as well as porcine MSP (pMSP) binds CRISPs present in the venoms from two Elapidae and two Viperidae snakes and provide a thorough characterization of the interactions.

2. Materials and methods

2.1. Materials

Crude venoms from *Crotalus atrox*, *Laticauda semifasciata*, *Pseudechis porphyriacus*, and *T. flavoviridis* were obtained as lyophilized powders (Sigma–Aldrich Corp., St. Louis, MO, USA). Human and porcine MSPs were purified from human and boar seminal plasma, respectively, with the method described for pMSP (Fernlund et al., 1994). MSP affinity columns were prepared by coupling about 8 mg of hMSP to a 1 ml HiTrap NHS-activated HP column (GE Healthcare BioSciences AB, Uppsala, Sweden) according to the instructions of the manufacturer. The coupling yields, calculated from the differences in concentrations of hMSP (determined by HPLC) in the coupling solution and the wash solution, were about 90%.

2.2. Purification of venom CRISPs

One hundred mg of lyophilized crude venom was extracted at room temperature with gentle shaking for 15 min in 5 ml of a 50 mM sodium phosphate buffer pH 7.4 containing 0.1 M NaCl. After centrifugation at 10,000 × *g* for 30 min, the clear supernatant was passed through a Minisart 0.20 μm filter (Sartorius AG, Goettingen, Germany) and then pumped through an hMSP-affinity column. The column was washed, first with 10–20 ml of a 50 mM sodium phosphate buffer pH 7.4 containing 0.1 M NaCl and then with 10–20 ml of the same buffer but with 0.5 M NaCl. Finally the bound material was eluted with 0.2 M glycine pH 2.0. The flow rate was 0.25 ml/min throughout the experiment. The absorbance of the effluent was continuously monitored at 280 nm with a 2238 Uvicord SII (LKB, Bromma, Sweden) and the part corresponding to the peak of absorbance was collected (usually about 2–3 ml), diluted to 6 ml with 50 mM sodium phosphate buffer pH 7.4 containing 0.5 M NaCl, and concentrated to 500 μl in a Vivaspin 6 ultra filtration spin column fitted with a 5 kDa cut off membrane (Vivascience Sartorius AG, Goettingen, Germany). The concentrated fraction was subjected to a final purification step by chromatography on a Superdex 75 10/300 GL column (GE Healthcare Biosciences AB), essentially as described for the analysis of complex formation (Section 2.4).

2.3. SDS-PAGE and protein identifications by mass spectrometry

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under reducing conditions according to Laemmli (1970) in a 4% (w/v) stacking, 12% (w/v) separating polyacrylamide gel. The gel was stained with GelCode blue stain reagent (Pierce, Rockford, IL, USA). The molecular size marker, Low Molecular Weight-SDS, was obtained as a ready-made mixture (Amersham Biosciences AB, Uppsala, Sweden). For