Identification of α-2u globulin in the rat preputial gland by MALDI-TOF analysis

P Ponmanickam and G Archunan*
Department of Animal Science, Bharathidasan University
Tiruchirappalli 620 024, TN, India

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The low molecular mass proteins found in the pheromonal sources such as urine, saliva, glandular secretion etc have been reported as ligand carriers for the processes of chemical communication in mammals. The preputial gland plays an important role in the production of olfactory signals for pheromonal communication. Thus, in the present study, α-2u globulin having molecular mass of 18 kDa has been identified in the preputial gland of Norway rat (Rattus norvegicus) by in-gel trypsin digestion and analyzing the resulting peptides by MALDI-TOF. Since preputial gland is one of the major pheromonal sources in rat, the results suggest that α-2u globulin might act as a carrier for hydrophobic odorants of preputial gland.

Keywords: Pheromones, Preputial gland, Binding protein, Lipocalin, Rat, MALDI-TOF

Material and Methods

Adult male Norway rats (Rattus norvegicus) were reared under laboratory conditions (light on from 06.00 to 18.00 h, temperature 24±1°C) and fed with rat feed (Sai Durga feeds, Bangalore) and water ad libitum. The bedding material was changed twice a week. Animals were sacrificed by cervical dislocation and preputial gland was excised and homogenized with PBS (pH 7.2).

Total proteins in the preputial gland estimated by the method of Bradford. One-dimensional SDS-PAGE was performed as described by Laemmli. The 50 µg protein was loaded on the gel. For determination of molecular mass, 4 µl of protein standard (protein molecular weight marker-medium range, Genei, Bangalore), was applied on the gel.

For MALDI-TOF analysis, the sample was prepared as described. Protein spot of 18 kDa was excised from SDS-PAGE and destained using 100 µl of 50 mM ammonium bicarbonate, 50% acetonitrile (trypsin), 50% acetonitrile (endopeptidase Lys C), and was incubated at 37°C for 30 min. This step was repeated until no stain was visible. The plugs were

FOR-efficient transport in aqueous body secretions, pheromone bind to proteins known as lipocalins (pheromone-binding proteins). Lipocalins are extracellular proteins with a molecular mass of ~20 kDa, display binding with high selectivity and affinity for small hydrophobic molecules. The physiological role of several lipocalins has been associated with the transport of hydrophobic compounds in aqueous biological fluids. Pheromone-binding proteins were reported in the urine of mouse and rat. They are known as major urinary proteins (MUP) in mice and α-2u globulin in rat. The ligand (odorant molecules)-binding property of MUP has been studied earlier and α-2u globulin plays a role in pheromone transport. The protein identified in hamster vaginal fluid is reported to act both as pheromone as well as its carrier. However, the information about pheromone-binding proteins in glandular sources of rodents is lacking. Hence, in the present study, an attempt has been made to identify α-2u globulin in the preputial gland of rat by MALDI-TOF and Mascot search.

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then incubated with 50 µl of 10 mM DDT. After 30 min at 37°C, the DDT was discarded and 50 µl of 55 mM iodoacetamide was added to each tube and incubated for 1 hr at room temperature in the dark. Thereafter, iodoacetamide solution was discarded and the plugs were washed twice with diethyl ether, dehydrated in 100% acetonitrile and then rehydrated in 9 µl of 50 mM ammonium bicarbonate (trypsin) and 1 mM EDTA (pH 8.5). Then, trypsin was added for converting protein to peptides and incubated overnight at 37°C.

After tryptic digestion, the mixture of peptides was placed in the MALDI target plate and mixed with the matrix solution. After calibration of known peptides, the samples were processed and mono-isotopic masses of spectra from the tryptic digested peptides were acquired for database searching. Based on the results, matching compound and the sequence coverage of the particular sample were obtained. Statistical evaluation of the results and scoring algorithms using Mascot (Matrix Science Ltd, http://www.matrixscience.com) facilitated identification of the best match.

Results and Discussion

In SDS-PAGE profile of preputial gland the low molecular mass protein i.e., 18 kDa appeared prominently. The presence of proteins with different molecular masses such as 158.3, 143.3, 90.2, 79.0, 63.0, 53.2, 47.7, 44.8, 42.7, 39.1, 36.3, 30.2, 29.2 and 27.7 kDa is also reported (Fig. 1).

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of 18 kDa protein in the preputial gland is consistent with earlier reports of the presence of similar protein in the preputial gland of Sprague-Dawley rat (R. norvegicus) and house rat (R. rattus) and urine of mouse (Mus musculus) and rat (R. norvegicus).

Mass spectrum of 18 kDa protein (Fig. 2) was obtained by MALDI-TOF and the mono isotopic number of spectrums were scored and analyzed with MASCOT search. Results of MASCOT search showed the presence of α-2u globulin in the first hit in the search list and the score was higher than the significant level (significant level was 76). In addition, the protein showed matching of maximum 10 peptides to rat urinary α-2u globulin, representing sequence coverage of 62% (Table 1). Similar binding protein called major urinary protein (MUP) has been identified in mouse urine from 19 kDa protein spot. Our results are consistent with this report.

Earlier, the pheromaxein (20 kDa) that can bind with steroid pheromones has been purified from porcine sub-mandibular gland. Another protein aphrodisin, secreted in hamster vaginal fluid by vaginal tissue and Bartholin gland, was also reported to be a pheromone carrier and facilitated the male copulatory behaviour. Apolipoprotein D (apoD), pheromone-binding protein in human carries the apocrine odour to skin surface which can alter the length and timing of the menstrual cycle. The relationship of volatile ligands such as brevicomin and thiazole with mouse urinary proteins and 2-isobutyl-3-methoxy pyrazine with rat urinary

Fig. 2—MALDI-TOF mass spectrum of rat preputial glandular protein [Protein spot from 18 kDa was subjected in-gel digestion with trypsin as described in the text]
proteins has been proved. The soluble proteins viz., urinary, sweat, vaginal and salivary proteins belong to a large family of lipocalins, and normally act as carriers for hydrophobic ligands. The present study provides the evidence of the presence of α-2u globulin, a pheromone-binding protein from rat preputial gland and its sequence coverage.

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