Volume 212, number 2, 225–228 FEB 04440 February 1987

Homology between the pyrazine-binding protein from nasal mucosa and major urinary proteins

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Received 14 November 1986

Sequence analysis of the pyrazine-binding protein from bovine olfactory mucosa reveals marked homology with a family of proteins of unknown function found in the urine of the adult male mouse and rat. In view of the dramatic biological responses to odorants transmitted in male rodent urines, it is proposed that these proteins play important roles in some aspects of odor transmission and reception.

Pyrazine-binding protein; Odor reception; Odor transmission; Olfaction

1. INTRODUCTION

No odorant-binding molecule has so far been identified as an olfactory receptor although proteins have been shown to be involved [1–3]. Recent reports of high-affinity odorant binding in olfactory mucosa [4–7] are, however, symptomatic of marked progress in the field. Pyrazine-binding protein [8,9] is synthesised in the cells of the seromucous tubulo-acinar glands of the nasal respiratory epithelium [10,11] of the cow, rabbit, pig, rat and mouse [12]. Although of unknown biological function, this protein has high-affinity association constants (10^6–10^9 M^{-1}) for a wide range of low-threshold but chemically unrelated odorants, some of which are characteristic of 'green' smeels [13]. Reasoning that the molecular mechanism of odor binding may be quite general, we have initiated the determination of the structure of bovine pyrazine-binding proteins. Here, we report a partial amino acid sequence which reveals a surprising homology with a group of urinary proteins of previously unknown function. A conserved region of about 30 residues suggests a common functional domain and may provide clues to the biological roles of both proteins.

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2. MATERIALS AND METHODS

Pyrazine-binding protein was extracted from bovine olfactory and respiratory mucosa according to Bignetti et al. [8] and purified as reported in [14]. Microcrystals of pyrazine-binding protein, prepared by cooling the protein solution on ice and adding ethanol to 20% (v/v), were stored at 4°C. Alkylation was carried out at 37°C in 0.3 M Tris-HCl (pH 8.6), 20 mM dithiothreitol using 50 mM iodoacetic acid. 1–5 nmol of the alkylated protein was dissolved in 0.1 ml of 67 mM Na phosphate buffer (pH 7.0) and incubated with 1.5% (w/w) *S. aureus* V8 protease for 3 h, at 37°C, modified from [15]. The reaction was terminated by freezing and lyophilisation.

For sequencing, lyophilised protein was dissolved in 0.07 ml of 0.2 M $NaHCO_3$/0.25% (w/v) SDS and added to 20 mg DITC-glass (preparation based on [16]). The glass was incubated for 90 min

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00145793/87/$3.50

at 56°C under N_2, then washed with water and methanol to remove non-covalently bound material. The glass-coupled protein was sequenced by automated solid-phase Edman degradation [17] using a high-sensitivity machine constructed in Leeds [18,19]. PTH-amino acids were identified by C18 reverse-phase HPLC using a method modified from [20]. The PTH-amino acids were quantitated by their absorbances at 265 nm and serine and threonine residues confirmed by detection of their dehydro derivatives at 313 nm.

3. RESULTS AND DISCUSSION

Amino acid sequencing of native protein indicated that the N-terminus was blocked, a yield of about 300 pmol PTH-amino acids being obtained from approx. 5 nmol coupled protein. It is not yet clear whether this reflects a true in vivo modification or artefactual blocking during the extraction and purification process. The yield was sufficient, however, to give an unambiguous 10-residue N-terminal sequence. Treatment of the native protein

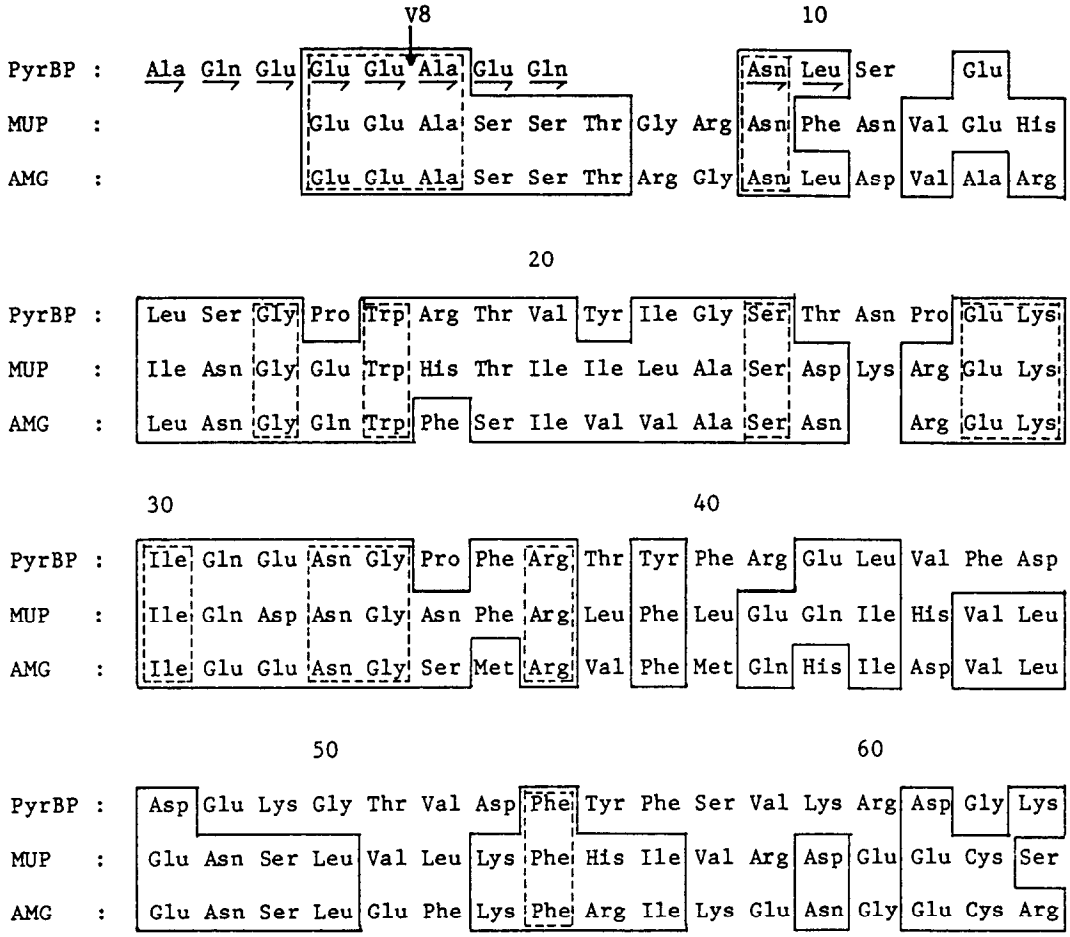

Fig.1. Initial sequence analysis of uncleaved protein provided a low-yield sequence of 10 residues (——→). Cleavage with *S. aureus* V8 protease (↓) specifically cleaved the N-terminal 5 residues. Sequence analysis of the cleaved protein (starting at Ala^6) was extended to 63 residues. The average repetitive sequencing yield was 94%. The program SEARCH was used to screen the NBRF protein sequence database (release 9.0).

with *S. aureus* V8 protease specifically and quantitatively removed the N-terminal 5 residues, generating a new N-terminus and allowing sequence analysis to be extended to 63 residues (fig.1). The average repetitive sequencing yield was 94%.

The sequence data on the N-terminal third of bovine pyrazine-binding protein (PyrBP) reveal a strong homology with the mouse major urinary proteins (MUP) and the rat α_{2u}-globulins (AMG) found in urine and in salivary glands [21–23] (fig.1). Neither group of proteins as yet has a defined function. Considering that the pyrazine-binding protein was obtained from bovine respiratory mucosa, the homologies are particularly strong, indicating greater than 30% identity (especially between residues 13 and 39) and nearly 60% similarity in residues. These figures, moreover, are a conservative estimate since other members of each family have substitutions similar to those seen in pyrazine-binding protein. This strong homology, occurring over a large portion (approx. 20%) of the proteins and across a substantial phylogenetic distance, is reinforced by the great similarity in molecular mass (18–20 kDa) and may point to a similarity in function.

In this respect we are reminded of the dramatic sexual effects aroused in mice and rats by adult male urine odor [24]. These effects are both behavioural (e.g. sexual attraction [25] and aggression [26]) and hormonal (e.g. puberty acceleration [27,28] and pregnancy blockage [29–31]). Also interesting is the observation that the synthesis of these urinary proteins is controlled by androgens and thus they are found only in the adult male [32]. It is possible, therefore, that these urinary proteins act as carriers of volatile pheromones. Certainly, there is evidence that the urinary component responsible for accelerating puberty is 'androgen-dependent, heat-labile, non-dialysable and precipitated by ammonium sulphate' [33].

The case for believing that these proteins act as some kind of odor carriers is strengthened by the observation that both the urinary polypeptides belong to large gene families (possessing at least 35 different members) and are highly conserved in phylogeny [34,35]. This would provide for the necessary flexibility and specificity required for odor binding [1]. The occurrence of conserved domains in bovine pyrazine-binding protein, mouse MUP and rat AMG suggests an involvement in the binding of similar molecules. It is thus important to examine the biological relevance of the suggested odor-carrier function for the urinary proteins. There is also the interesting possibility that at least some of the olfactory receptor proteins might be expressed members of this gene family.

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