Bovine parotid secretory protein: structure, expression and relatedness to other BPI (bactericidal/permeability-increasing protein)-like proteins


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Abstract

Members of the family of BPI (bactericidal/permeability-increasing protein)-like proteins are as yet incompletely characterized, particularly in cattle, where full-length sequence information is available for only three of the 13 family members known from other species. Structural bioinformatic analyses incorporating bovine homologues of several members of the BPI-like protein family, including two forms of bovine parotid secretory protein (PSP), showed that this family is also present in cattle. Expression analyses of several members of the BPI-like protein family in cattle, including PSP (Bsp30), von Ebner’s minor salivary gland protein (VEMSGP) and lung-specific X protein (LUNX), showed a restricted pattern of expression, consistent with earlier hypotheses that these proteins function in the innate immune response to bacteria. The possible role of bovine PSP in susceptibility to pasture bloat in cattle is discussed.

Introduction

The BPI (bactericidal/permeability-increasing protein)-like family of proteins (also referred to as the lipopolysaccharide binding protein (LBP), lung-specific X protein (LUNX)/palate, lung and nasal epithelial clone (PLUNC) and parotid secretory protein (PSP)/LBP family [1–3]) comprises at least 13 structurally related proteins, most of which have been identified only recently as a result of genome sequence analysis. These proteins are characterized by containing either one or two BPI domains within their amino acid sequence. The genes for all except two of these proteins are located close together as a cluster on human chromosome 20 [2–4].

Each of the members of the BPI-like family appears to have a distinct pattern of expression in tissues. For example, BPI is found in leucocyte secretory granules; LBP, phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) are plasma proteins produced predominately by the liver; PSP (also known as SPLUNC2 [3]) is expressed in salivary glands; and LUNX (also known as Plunc or SPLUNC1 [3,5]) is expressed in airway epithelia [1,6–8]. However, the patterns of expression of several members of the gene family, including von Ebner’s minor salivary gland protein (VEMSGP; also known as LPLUNC1 [3]), RY2G5 (also known as LPLUNC4 [3]), RYA2 (also known as LPLUNC3 [3]), protein X (also known as SPLUNC3 [3]), protein Y (also known as BPI3 [4]) and protein Z (also known as LPLUNC2 or BPIL1 [3,4]), have not been very well characterized.

Very little is known about the functions of most of the BPI-like family members. The three-dimensional structure of one member of the family (BPI) is known, revealing a hydrophobic pocket in each BPI domain [9]. The BPI domains in other members of the family are also known to bind hydrophobic molecules, including lipopolysaccharide (LPS) (e.g. BPI and LBP), phospholipids (PLTP) and cholesterol (CETP) [1,6]. Two members of the family (RYA3 and RY2G5) that are expressed in rat olfactory tissue have been proposed to bind to small hydrophobic odorants [10]. The members of the BPI family appear to function in a wide range of biological contexts, including plasma lipid transport, the inflammatory response and immune defence. However, for most of the family members, the proposed biological function awaits experimental verification.

The BPI-like proteins are relatively uncharacterized in species other than humans. Our research has led to the identification of two closely related homologues of one family member, PSP, in cattle. These proteins, which we term Bsp30a and Bsp30b, are highly expressed in the parotid salivary gland and comprise approx. 30% of the protein in bovine saliva [11]. Here we show the relationship of the two bovine PSP proteins (Bsp30a and Bsp30b), as well as some additional family members we have found in cattle, with their
homologues from other species. In addition, we show the pattern of expression of some of these family members in bovine tissues.

**Bioinformatic analysis of the BPI-like family**

The amino acid sequences of known members of the BPI-like family were obtained from public sequence databases using a series of BLAST and PSI-BLAST analyses, as described previously [2]. These are defined through sequence similarity (up to 43% identity between family members) and a similar pattern of exon segmentation [3]. The family members can be divided into two groups: one group comprises proteins of approx. 250 amino acids and containing a BPI1 domain, and the other group comprises proteins of approx. 530 amino acids and containing BPI1 and BPI2 domains. In humans, the genes for 11 of the 13 known family members are present as a cluster on chromosome 20q11.2, and homologues for 10 of these are present on mouse chromosome 2. The genes for eight of the family members (VEMSGP, LUNX, protein X, PSP, RY2G5, RYA3, protein Y and protein Z) are contiguous on the human chromosomal sequence [2,3].

To identify BPI-like proteins in cattle, BLAST analyses were performed against public databases as well as an in-house database of over 200,000 expressed sequence tags (ESTs) from a range of bovine tissues. The results reveal that at least partial sequence coding for bovine homologues of 11 of the 13 known members of the BPI-like family is present in cattle, with only RYA3 and CETP absent. A multiple sequence alignment was performed of all available full-length amino acid sequences using the Vector NTI program, and a phylogenetic tree was constructed. The results confirm that the bovine sequences are indeed homologues of their human, murine and rat counterparts (Figure 1). The inability to detect bovine homologues of RYA3 and CETP in the EST database could be a consequence either of the incomplete representation of expressed genes in the database or of the fact that these genes are not present in cattle. Further work will be required to distinguish between these possibilities.

Interestingly, in cattle there are two very closely related forms of PSP (83% identity at the amino acid level), whereas both human and mouse appear to have only one form of PSP. This observation illustrates that significant differences occur between some species with regard to the BPI-like family. Our previous work showed that the two forms of PSP are present in bovine genomic DNA from all animals tested, and that therefore they are very likely to arise from two separate genes [12]. It is not known whether the genes encoding these proteins are clustered together in the bovine genome, and in particular whether the two bovine PSP genes are contiguous, as might be expected if they arose from a duplication event. Confirmation of this awaits the availability of bovine genomic sequence of the gene locus. In addition to PSP, another PSP-like protein is expressed in rats, called SMGB (submandibular gland protein B). However, amino acid sequence analysis suggests that it is unlikely to be a homologue of Bsp30b. PSP and SMGB, like bovine Bsp30a and Bsp30b (see below), are expressed independently of one another.

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**Figure 1**  |  Phylogenetic tree derived from alignment of the family of BPI-like proteins using the Vector NTI program (Accelrys)

Bovine, human, mouse and rat proteins are annotated b, h, m and r respectively. GenBank identifiers for the sequences are: Bsp30a, AAB38282; Bsp30b, AAB38283; human PSP, AAL28113; mouse PSP, P07743; rat PSP, B42337; rat SMGB, AAC12783; bovine VEMSGP, AAM00906; human VEMSGP, NP_149974; mouse VEMSGP, AAAB7581; bovine PLUNC, AAM00907; human PLUNC, BAA93633; mouse PLUNC, AAB63256; rat PLUNC, AAM73687; human RYA3, CAC18886; rat RYA3, CAA43065; bovine RY2G5, B754700; human RY2G5, CAC18887; rat RY2G5, CAA43067; human protein Z, CAC18884; mouse protein Z, NP_079907; human protein Y, XP_066207; bovine BPI, P17453; human BPI, AAB31144; mouse BPI, BAC28468; human LBP, NP_004130; mouse LBP, NP_032515; rat LBP, NP_058904; human PLTP, NP_006218; mouse PLTP, AAB87943; human CETP, NP_000069; human protein X, XP_059283; mouse protein X, BAB24760. Bovine LBP and protein Z protein sequences were obtained from the AgResearch in-house EST database. Mouse RY2G5 and RYA3 protein sequences were obtained by manual assembly of exon nucleotide sequence produced by a BLAST search of the UCSC mouse genome database with the appropriate rat homologue amino acid sequence, followed by translation.
Expression of BPI-like proteins in cattle

Our earlier studies showed that the two forms of bovine PSP (Bsp30a and Bsp30b) are highly expressed exclusively in the major salivary glands of cattle, and that Bsp30a and Bsp30b appear to be expressed independently of one another. In addition, bovine LUNX/PLUNC was found to be highly expressed in trachea, while bovine VEMSGP was expressed only at low levels in parotid gland, nasopharyngeal tissues, and trachea [2]. These earlier studies were limited in that they did not include several tissues of interest for the expression of proteins involved in host immune response. Furthermore, the extent of inter-animal variability could not be assessed accurately, as only two individual cattle were analysed.

RNA from a more extensive range of bovine salivary, lymphatic and pharyngeal tissues was analysed by Northern blotting in order to determine whether these proteins are expressed in additional tissues associated with a function in host defence. In addition to salivary gland expression, Bsp30b was expressed at low levels in the inner cheek lining (Figure 2). Both VEMSGP and LUNX/PLUNC were expressed in the nasal epithelium and trachea, although VEMSGP was also expressed in the parotid salivary gland and at very low levels in the inner part of the tongue. Notably, expression of Bsp30, LUNX/PLUNC and VEMGSP was not detected in the immune tissues examined (tonsil, pharyngeal lymph node) or in white blood cells, as distinct from BPI, indicating that their function is associated with the oral cavity and respiratory tract.

Towards a function for BPI-like proteins in cattle

Our initial interest in bovine PSP was sparked by an apparent correlation of its abundance in saliva with the degree of susceptibility of cattle to pasture bloat. Pasture bloat is an inherited trait based on 20 years of genetic selection applied in cattle [13]. Electrophoretic analysis of saliva from these selected cattle revealed a major salivary protein (Bsp30) whose abundance was increased by 66% in animals with a low susceptibility to bloat, with a correlation coefficient of $-0.40$. In order to determine the extent of variability of expression of Bsp30a and Bsp30b between animals, RNA was isolated from the left and right parotid glands of 10 cattle. The RNA was analysed for expression of Bsp30a and Bsp30b using oligonucleotide probes specific for each RNA, as described previously [2]. In addition, as a normalization control, the abundance of β-actin RNA was determined by Northern blotting in each of the samples (Figure 3). From a qualitative analysis, the levels of expression of both Bsp30a and Bsp30b appeared approximately equal between the left and right parotid glands from each animal. However, the absolute abundance of both Bsp30a and Bsp30b varied significantly between animals. Furthermore, the abundance of Bsp30a mRNA did not appear to be correlated with the abundance of Bsp30b mRNA in the same tissue. For instance, animals 6, 7 and 8 had similar levels of Bsp30a, yet animal 6 had no detectable Bsp30b mRNA. These results suggest that the expression of the bovine PSP genes is subject to physiological control (either genetically determined within each animal or subject to environmental influence), and that the expression of Bsp30a and Bsp30b is controlled independently. It is not possible to determine from these data what genetic or environmental factors may influence Bsp30a or Bsp30b expression.
The characterization of the family of BPI-like proteins is not yet complete. With the exception of BPI, for which three-dimensional structure, biochemical data and biological function are available, little information has been published beyond the primary structure and simple expression studies. In cattle, the BPI-like family has had limited attention. The present study has characterized the existence of members of this family in cattle, and demonstrated a restricted pattern of expression, similar to that in other species. Cattle provide a useful model organism with which to investigate the function of members of the BPI-like proteins, as relatively large amounts of tissues and secretions can be obtained, facilitating their purification as well as in-depth studies of their expression. It is possible that several members of the family for which a function is currently not yet proven may play a role in host defence/innate immunity. Further investigation of the BPI-like proteins in cattle may therefore help us to understand better the innate immune response to bacteria and/or the role of PSP in pasture bloat.

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References

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