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Quantification of Bovine Colostral Odorant-Binding Protein (bcOBP) mRNA Distributed in Principal Organs of Bovine Body

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ABSTRACT

A novel protein, bcOBP was found in bovine colostrums. This protein belongs to the lipocalin superfamily, most of them are secretory proteins. The aim of this study was to investigate organs that express bcOBP mRNA with its expression level and to determine the origin of bcOBP in the bovine body. We quantified bcOBP mRNA in several organs of bovine body with qPCR. The expression was found in the principal organs (heart, liver and kidney), node and mammary gland. In addition, the expression level of bcOBP in heart is as much as in kidney among principal organs. Further, *in situ* hybridization analysis opened the possibility that bcOBP in bovine colostrums might be secreted from the mammary alveolus in mammary gland. Our study provides a fundamental concept for lacto protein.

Key words: Lipocalin, colostrum, cow and colostrums protein, mammary gland

INTRODUCTION

Lipocalin superfamily shares highly conserved structures, whereas Amino Acid (AA) sequences are widely different in animals (Akerstrom *et al*., 2000). They show various biological functions, including retinol transport (Kanai *et al*., 1968), fatty acid transport (Cancedda *et al*., 1988) and immunomodulation (Rachmilewitz *et al*., 1999). Odorant-Binding Proteins (OBPs) belong to lipocalin superfamily and transport hydrophobic molecules such as odorants. Bovine OBP (bOBP), derived from bovine nasal mucosa, was first identified among mammals (Pevsner *et al*., 1985; Bignetti *et al*., 1985).

In 2009, a novel protein in bovine colostrum was found by Japanese research groups, which named bovine colostral Odorant-Binding Protein (bcOBP) (Fukuda *et al*., 2009). This protein consists of 172 AA residues, including a putative 16 AA signal peptide. OBP derived from nasal mucosa showed low sequences similarity (52%) to bcOBP. Our previous study indicated that bcOBP mRNA was expressed in bovine several tissues without its localization (Katayama and Japaridze, 2014). However, the expression level of bcOBP mRNA and the origin of bcOBP remain unknown. In this study, we attempted to identify the expression level of bcOBP mRNA in principal organs. Further, we investigated the reason why bcOBP was found in bovine colostrums.

MATERIALS AND METHODS

RNA isolation and reverse transcription: Total RNA was purified with Trizol reagent (Invitrogen) and treated with RQ1 RNase free DNase (Promega) to remove genomic DNA contamination. One microgram of total RNA was used for reverse transcription reaction with ReverTraAce (Toyobo) and random primer (Invitrogen), according to the manufacturer's instructions. Quantitative PCR was performed with Taqman RT-PCR using Premix Ex Taq (Takara) and analyzed with the 7300 real-time PCR system (Applied Biosystems).

Probe sequences used in quantitative PCR: FAM, 6-carboxyfluorescin; TAMRA, 6-carboxytetramethylrhodamine, β-actin.

Forward, 5'-TGCCATGTATGTGGCCATCC-3'; reverse, 5'-CGCTCGGCTGTGGTGGTAA-3'; probe, 5'-FAM-ACTCTGGGGACGGGGTCA-TAMRA-3', bcOBP.

Forward, 5'-GCACATGCCAGTTTTTCTCA-3'; reverse, 5'-GGAATCCCCCTCTCATTGTT-3'; probe, 5'-FAM-AAGGTTCCGCCAAAGGAACCA-TAMRA-3'.

Tissue collection and preparation: We used sick cows in lactation stage. The tissues without suspicion of pathological features were used. The tissues were fixed with 4% paraformaldehyde in PBS and embedded in paraffin for *in situ* hybridization. Serial sections were cut at 5 μm thickness and then placed onto 3-aminopropyltriethoxysilane-coated glass slides.

Probes and labeling: Oligo-DNA complementary to a part of bcOBP mRNA was synthesized together with additional three ATT repeats at the 3'-end and 5'-end and haptenized with DIG Oligonucleotide Tailing Kit (Roche), according to the manufacturer's instructions.

***In situ* hybridization:** Paraffin sections were deparaffinized and rehydrated with a toluene-ethanol series. The slides were treated with PBS for 10 min and proteinase K (10 μg mL^{-1}, 37°C, for 15 min), successively. After postfixation with 4% paraformaldehyde (PFA) in Phosphate Buffered Saline (PBS) (5 min), the sections were immersed in 2 mg mL^{-1} glycine in PBS (30 min) and kept in 40% deionized formamide in 4×SSC until used for hybridization. Hybridization was carried out at 42°C for 12-16 h with Dig-labeling oligo-cDNA for bcOBP dissolved in the hybridization medium. After repeated washings, the slides were incubated with POD-conjugated ovine anti-DIG Fab fragment (Roche). The sites of peroxidase activity were visualized with DAB substrate (Roche) according to the manufacturer's instructions.

RESULTS

Quantification of the expression of bcOBP mRNA: To quantitate the expression of bcOBP mRNA, we isolated mRNA from bovine organs. TaqMan quantitative PCR (qPCR) was performed for those samples. qPCR analysis revealed that the expression was found in the principal organs such as heart, liver, kidney, node and mammary gland (Fig. 1). In addition, the expression level of bcOBP mRNA in heart is as much as in kidney among principal organs (Fig. 1).

Localization of bcOBP mRNA in mammary gland: To investigate the origin of bcOBP, we detected bcOBP mRNA in mammary gland with *in situ* Hybridization (ISH). In mammary glands, the higher expression in the mammary alveolus than in supporting tissues of mammary glands has been observed (Fig. 2).

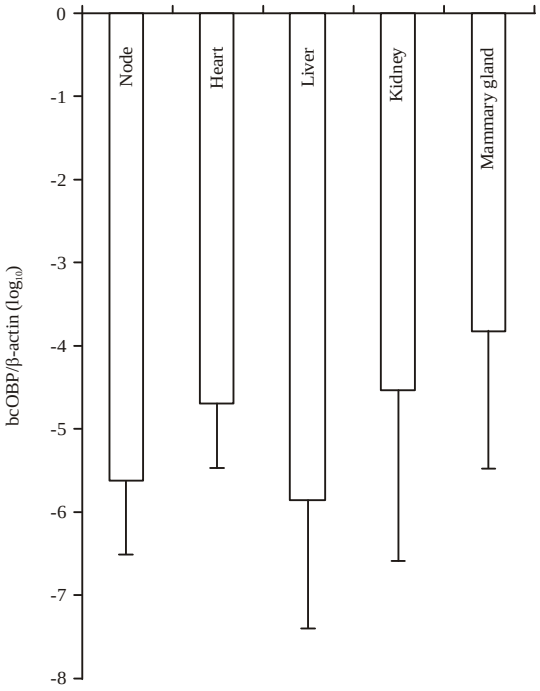

Fig. 1: Quantitative PCR analysis of bcOBP transcription in several tissues. Transcript levels were normalized to β-actin. Shown are the averages and Standard Derivations (SD) in three independent experiments

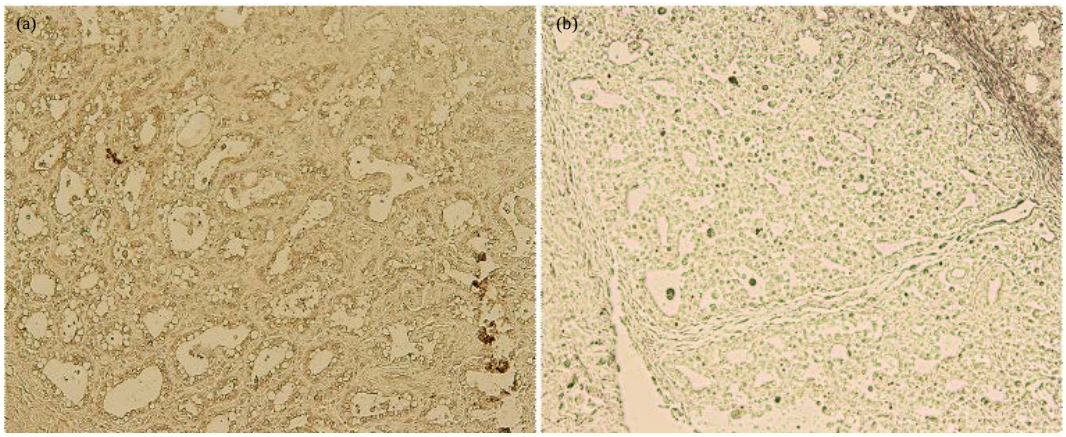

Fig. 2(a-b): Tissue section *in situ* detection of bcOBP mRNAs. (a) DIG-POD-DAB stained (×100) mammary gland sections were hybridized with digoxigenin-labelled bcOBP antisense probes and (b) Control (×100). No signal was obtained with sense probes used as negative control

DISCUSSION

In the present study, we discovered that bcOBP mRNA were expressed in principal organs, indicating that bcOBP might be involved in various physiological functions. Importantly, the

expression level of bcOBP mRNA in heart is as much as in kidney. This fact exhibits a possibility that bcOBP has important roles for maintenance of homeostasis. In human, two novel protein genes (hOBP a and hOBP b) were found (Lacazette *et al*., 2000). hOBP a had an oral sphere expression profile and hOBP b was expressed in genital sphere organs (Lacazette *et al*., 2000). They hypothesized that two hOBP had critical roles for maintenance of homeostasis in human body (Lacazette *et al*., 2000). However, the functional roles of bcOBP and two kinds of hOBP remain unknown. Our findings provide a fundamental concept for further study such as their biological functions.

bcOBP was found in bovine colostrums (Fukuda *et al*., 2009). No tight in the hypothesis and findings on the origin of bcOBP. There was a hypothesis that bcOBP found in bovine colostrums were secreted from mammary gland. Mammary gland, especially mammary alveolus, might secrete bcOBP into colostrums because higher expression of bcOBP mRNA in mammary alveolus than in supporting tissues (Fig. 2). This result supported the possibility that a bcOBP protein found in bovine colostrums was secreted from mammary alveolus in mammary gland. There is also a possibility that other organs which express bcOBP mRNA secret bcOBP protein to blood and then bcOBP-blood is converted to colostrums. Alpha-1-acid glycoprotein (AGP), found in human blood (Piafsky *et al*., 1978), is also a lipocalin in bovine milk (D'Amato *et al*., 2009). AGP in human blood is secreted from liver (Fournier *et al*., 2000). AGP in bovine milk is considered to be synthesized in mammary tissues and in somatic cells (Ceciliani *et al*., 2007). With this condition, no finding was determined.

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