NOTE *Molecular Biology*

Identification of Canine α-Lactalbumin

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ABSTRACT. The nucleotide sequence of canine α-lactalbumin cDNA from canine mammary tissue was determined by polymerase chain reaction with degenerate primers. A 742 base pairs nucleotide sequence cloned was similar to the size of mRNA in Northern blot analysis. The cDNA encodes 142 amino acid residues containing the conserved sequence motif of α-lactalbumin, demonstrating the highest homology with pig (73% identity-82% similarity) among the known amino acid sequences of α-lactalbumin. The canine cDNA also showed 71% identity-78% similarity with human, 58–73% with mouse, 60–74% with rat, 67–77% with goat, 66–77% with cattle, and 67–76% with sheep, respectively.

KEY WORDS: canine, degenerate oligo primer, α-lactalbumin.

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α-Lactalbumin is a calcium metalloprotein which is a major component of milk proteins [7]. In the Golgi apparatus of lactating mammary epithelial cells, α-lactalbumin promotes lactose synthesis by modifying the substrate specificity of uridine diphosphate (UDP)-galactosyltransferase (EC 2.4.1.22) so as to utilize glucose as an acceptor instead of terminal N-acetyl-glucosamine residues of glycoproteins [11].

The nucleotide and amino acid sequences of α-lactalbumin have been reported for various mammalians [3, 5, 6, 8, 9, 13, 14] but the canine α-lactalbumin sequence has not been published.

Here, we show molecular cloning of the canine α-lactalbumin cDNA and compare the primary structure of α-lactalbumin among various mammalian species.

We cloned the canine α-lactalbumin cDNA by using degenerate primer-based polymerase chain reaction (PCR) from canine mammary tissue. The nucleotide sequence of primers used for PCR was determined, based on the conserved nucleotide sequence of α-lactalbumin among human [5], mouse [14], rat [8], goat [6], cattle [13] and sheep [3] (laDOP-01; 5'-AGTGGITAIGACACACAAGC-3' and laDOP-02; 5'-CCACTGITCIAGCTTCTCAG-3'; I, inosine). PCR was performed as follows: 1 cycle at 95°C for 2 min, 40 cycles at 95°C for 30 sec, 44°C for 1 min, 72°C for 2 min, and 1 cycle at 72°C for 5 min. PCR products of about 250 base pairs (bp) were purified from an agarose gel and inserted into a pT7-blue vector (Novagen, WI). Nucleotide sequencing revealed that 18 of 24 clones tested shared an identical sequence, which was named cala cDNA.

To determine the full-length nucleotide sequence of the canine α-lactalbumin message, 5' and 3' rapid amplification of cDNA ends (RACE) was performed. 5' RACE was performed with the 5' RACE System for Rapid Amplification of cDNA Ends, Version 2.0 (5'AAP; 5'-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3' and AUAP; 5'-GGCCACGCGTCGACTAGTAC-3') (Gibco BRL, NY) and gene-specific primers generated on information from the nucleotide sequence of cala cDNA (cala-07; 5'-ACTGAAGGTTCTGGTCGTCCTTGC-3' and cala-08; 5'-TCCAGGAACTTGTCACAGGAGATGTC-3'). We determined the 5' nucleotide sequence of 203 bp. To determine the 3' end of the canine α-lactalbumin message, we synthesized first strand cDNA from canine mammary tissue with an oligo-dT adapter primer (5'-GCGGCTGAAGACGGCCTATGTGGCCTTTTTTTTTTTTTTTT-3'). With the first strand cDNA, we obtained a 326-bp nucleotide sequence of the 3' end with an anchor primer (705; 5'-GCGGCTGAAGACGGCCTATGT-3') and gene-specific primers (cala-09; 5'-GTGACATCTCCTGTGACAAGTTCCTG-3' and cala-10;

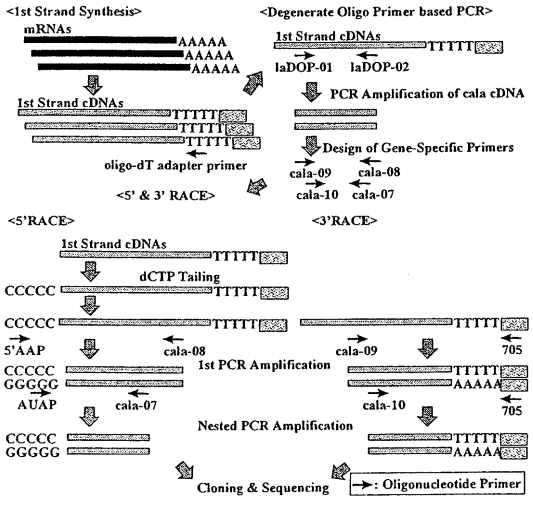

Fig. 1. Scheme of the cloning of canine α-lactalbumin.

A

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dog          : MMSFVSLLLVSIL-FPAIQA  19
pig          : MMSFVSLLVVGIL-FPAIQA  19
d-yak        : MMSFVSLLLVGIL-FHATQA  19
cattle       : MMSFVSLLLVGIL-FHATQA  19
w-buffalo    : MMSFVSLLLVGIL-FHATQA  19
goat         : MMSFVSLLLVGIL-FHATQA  19
sheep        : MMSFVSLLLVGIL-FHATQA  19
human        : MRFFVPLFLVGIL-FPAILA  19
g-pig        : MMSFFPLLLVGIL-FPAVQA  19
mouse        : MMHFVPLFLVCILSLPAFQA  20
rat          : MMRFVPLFLACIS-LPAFQA  19
rabbit       : MMPLVPLLLVSIV-FPGIQA  19
c-possum     : MMSLLPLLLIGIV-LPATQA  19
t-wallaby    : MMSLLSLLLLGIA-LPATQA  19
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B

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                                                box1                box2
dog          : KQFTKCELPQVLKD--MDGFGGIALPEWICTIFHTSGYDTQTIVNNNGGTDYGLFQISNKFWCKDDQNLQ---SRN  90
pig          : KQFTKCELSQVLKD--MDGYGDITLPEWICTIFHISGYDTKTIVHDNGSTEYGLFQINNKLWCRDNQIQ----SKN  89
a-camel      : KQFTKCKLSDELKD--MNGHGGITLAEWICIIFHMSGYDTETVVSNNGNREYGLFQINNKIWCRDNENLQ---SRN  71
horse        : KQFTKCELSQVLKS--MDGYKGVTLPEWICTIFHSSGYDTQTIVKNNGKTEYGLFQINNKMWCRDNQILP---SRN  71
donkey       : KQFTKCELSQVLKS--MDGYKGVTLPEWICTIFHSSGYDTQTIVKNNGKTEYGLFQINNKMWCRDNQILP---SRN  71
d-yak        : EQLTKCEVFRELKD--LKGYGGVSLPEWVCTTFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPH---SSD  91
cattle       : EQLTKCEVFRELKD--LKGYGGVSLPEWVCTTFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPH---SSN  91
w-buffalo    : EQLTKCEVFRELKD--LKDYGGVSLPEWVCTTFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPH---SSN  91
goat         : EQLTKCEVFQKLKD--LKDYGGVSLPEWVCTAFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPH---SRN  91
sheep        : EQLTKCEAFQKLKD--LKDYGGVSLPEWVCTAFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPH---SRN  91
human        : KQFTKCELSQLLKD--IDGYGGIALPELICTMFHTSGYDTQAIVENNESTEYGLFQISNKLWCKSSQVPQ---SRN  91
y-baboon     : KQFTKCELSQNLYD--IDGYGRIALPELICTMFHTSGYDTQAIVENDESTEYGLFQISNALWCKSSQSPQ---SRN  71
g-pig        : KQLTKCALSHELND--LAGYRDITLPEWLCIIFHISGYDTQAIVKNSDHKEYGLFQINDKDFCESSTTVQ---SRN  90
mouse        : TELTKCKVSHAIKD--IDGYQGISLLEWACVLFHTSGYDTQAVVNDNGSTEYGLFQISDRFWCKSSEFPE---SEN  91
rat          : TEFTKCEVSHAIED--MDGYQGISLLEWTCVLFHTSGYDSQAIVKNNGSTEYGLFQISNRNWCKSSEFPE---SEN  90
rabbit       : TQLTRCELTEKLKE--LDGYRDISMSEWICTLFHTSGLDTKITVNNNGSTEYGIFQISDKLWCVSKQNPQ---SKN  90
echidna      : KVFEKCELSQMLKANGLDGFQGITLEEWICIAFHESGFDSRALNYYNGSSSHGLFQINRQYWCDGQDAKSTEPSVN  76
d-platypus   : RIFQICELSRVLKENGLGGFHGVSLEEWLCVIFHESGYDSQALNYYNGSSSHGLFQINQPYWCDDXDSESTEPSVN  76
c-possum     : KDYGKCELNQILRE--RGVDKVISLPELICTMFHSSGFSTETEVDNNNHKEYGIFQISSNGWCAEKQEDV---ERS  90
t-wallaby    : IDYRKCQASQILKE--HGMDKVIPLPELVCTMFHISGLSTQAEVNNHSNKEYGIFQISNNGWCAEKQEDV---ANS  90
                     *     :           :.: * *  ** ** .:  .        .:*:***.    :*          .

                box3                          box4      box5
dog          : ICDISCDKFLDDDLTDDMICAKKILDK-EGIDYWLAHKPLCSEKLDQWRCEKL----------------  142
pig          : ICGISCDKFLDDDLTDDMMCAKKILDN-EGIDYWLAHKALCSEKLDQWLCEKM----------------  141
a-camel      : ICDISCDKFLDDDLTDDKMCAKKILDK-EGIDYWLAHKPLCSEKLEQWQCEKW----------------  123
horse        : ICGISCDKFLDDDLTDDVMCAKKILDS-EGIDYWLAHKPLCSEKLEQWLCEEL----------------  123
donkey       : ICGISCNKFLDDDLTDDVMCAKKILDS-EGIDYWLAHKPLCSEKLEQWLCEEL----------------  123
d-yak        : ICNISCDKFLDDDLTDDIMCVKKILDK-VGINYWLAHKALCSEKLDQWLCEKL----------------  142
cattle       : ICNISCDKFLDDDLTDDIMCVKKILDK-VGINYWLAHKALCSEKLDQWLCEKL----------------  142
w-buffalo    : ICNISCDKFLDDDLTDDIMCVKKILDK-VGINYWLAHKALCSEKLDQWLCEKL----------------  142
goat         : ICNISCDKFLDDDLTDDIVCAKKILDK-VGINYWLAHKALCSEKLDQWLCEKL----------------  142
sheep        : ICNISCDKFLDDDLTDDIVCAKKILDK-VGINYWLAHKALCSEKLDQWLCEKL----------------  142
human        : ICDISCDKFLDDDITDDIMCAKKILDI-KGIDYWLAHKALCTEKLEQWLCEKL----------------  142
y-baboon     : ICDITCDKFLDDDITDDIMCAKKILDI-KGIDYWIAHKALCTEKLEQWLCEKE----------------  123
g-pig        : ICDISCDKLLDDDLTDDIMCVKKILDI-KGIDYWLAHKPLCSDKLEQWYCEAQ----------------  142
mouse        : ICGISCDKLLDDELDDDIACAKKILAI-KGIDYWKAYKPMCSEKLEQWRCEKP----------------  143
rat          : ICDISCDKFLDDELADDIVCAKKIVAI-KGIDYWKAHKPMCSEKLEQWRCEKPGAPALVVPALNSETPVP  159
rabbit       : ICDTPCENFLDDNLTDDVKCAMKILDK-EGIDHWLAHKPLCSENLEQWVCKK----------------  141
echidna      : ACQISCDKLRDDDIEDDIKCVKKILKESQGITAWEAWQPFCIADLDQWKC------------------  126
d-platypus   : ACQIPCSKLLDDDILDDIECAKKIVKEPKGITAWEAWQPFCNSDLDQWKC------------------  126
c-possum     : VCGILCSKLLDDDITDDIVCAKKILQLPERLDHWKAHNTFCRENLDQWNC------------------  140
t-wallaby    : VCGILCSKFLDDDITDDIECAKKILQLPEGLGYWKAHETFCLEDLDQWRC------------------  140
                *   *.:: **:: **  *  ** :    :  * * :.:*   .*:** *
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Fig. 2. Alignment of the amino acid sequence of the mammalian α-lactalbumin genes. The Genbank accession numbers are as follows: AB035079 (dog; *Canis familiaris*), P18137 (pig; *Sus scrofa*), P00710 (a-camel: arabian camel; *Camelus dromedarius*), P08334 (horse; *Equus caballus*), P28546 (donkey; *Equus asinus*), AAF06793 (d-yak: domestic yak; *Bos grunniens*), P00711 (cattle; *Bos taurus*), AAF06794 (w-buffalo: water buffalo; *Bubalus arnee bubalis*), P00712 (goat; *Capra hircus*), P09462 (sheep; *Ovis aries*), P00709 (human; *Homo sapiens*), 1ALC (y-baboon: yellow baboon; *Papio hamadryas cynocephalus*), P00713 (g-pig: guinea pig; *Cavia porcellus*), NP_034809 (mouse; Mus musculus), NP_036726 (rat; *Rattus norvegicus*), AAD56598 (rabbit; *Oryctolagus cuniculus*), P81646 (echidna; *Tachyglossus aculeatus aculeatus*), P30805 (d-platypus: duckbill platypus; *Ornithorhynchus anatinus*), Q29145 (c-possum: common brush-tailed possum; *Trichosurus vulpecula*), A60394 (t-wallaby: tammar wallaby; *Macropus eugenii*). The alignment was made with the CLUSTALW program via the internet (http://www.ddbj.nig.ac.jp/htmls/E-mail/clustalw-j.html). An asterisk (*), an identical residue; A colon (:), a very similar residue; and a period (.), a less similar residue. Conserved regions of protein sequence are boxed (boxes 1–5).

5'-TGGTTGGCCCATAAACCACTCTGC-3'). The entire canine α-lactalbumin cDNA consisted of a 742-bp nucleotide sequence (Fig. 1).

The nucleotide sequence of the α-lactalbumin cDNA (accession No. AB035079) showed that the start codon was at nucleotide 28–30 and the stop codon was at nucleotide 454–456. The canine α-lactalbumin cDNA contained an open reading frame of 426 bp encoding a putative polypeptide of 142 amino acids, including a signal sequence of 19 residues.

About 0.8 kb mRNA was detected in canine mammary tissues by Northern blot analysis with cala cDNA as a probe, indicating that the 742 bp nucleotide sequence represents an almost entire canine α-lactalbumin cDNA (data not shown).

Comparison of the deduced amino acid sequence of canine α-lactalbumin with the database sequence by means of the BLASTP program [1] revealed that the canine sequence had the highest homology with porcine α-lactalbumin (73% identity-82% similarity). The canine polypeptide also had homologies comparable to those of horse and donkey (72% identity-80% similarity). Moreover, homology of the predicted canine α-lactalbumin polypeptide with other mammalian α-lactalbumin was 71% identity-78% similarity with human, 58–73% with mouse, 60–74% with rat, 67-77% with goat, 66–77% with cattle, and 67–76% with sheep, respectively.

To further compare the amino acid sequence among mammalians in detail, a multiple alignment was made by means of the CLUSTALW program [12]. As shown in Fig. 2, the alignment of the α-lactalbumin amino acid sequence for 20 species (premature sequence for 14 species and mature one for 6 species), revealed that the overall structure of the amino acid sequence was similarly conserved. Among the premature amino acid sequences of 14 species, 19 or 20 residues for the predicted signal peptide were highly conserved (Fig. 2A).

Among the mature α-lactalbumin amino acid sequences of 20 species, we found several conserved motifs (Fig. 2B). The alignment of these proteins also revealed that the α-lactalbumin/lysozyme C signature, which corresponds to a calcium binding loop [10], was well conserved (box 3). Phe-His-X-Ser-Gly-X-Asp/Ser-Thr/Ser (X: any amino acid) (box 1) and X-Leu-Asp/Glu-Gln-Trp-X-Cys (box 5), which are related to the interaction of α-lactalbumin with UDP-galactosyltransferase [10], were also well conserved. Besides the known motifs within α-lactalbumin, we found several conserved peptide sequences. Gly-Leu/Ile-Phe-Gln-Ile, (box 2), was conserved for all species. Trp-X-Ala-$(X)_4$-Cys (box 4) was also similarly conserved.

Lysozyme has been shown to be structurally related to α-lactalbumin and it has been proposed that these proteins originate in a common ancestral gene [2]. We evaluated the hypothesis in the canine case. A comparison between canine α-lactalbumin and canine lysozyme C (EC3.2.1.17) (accession No. S48641) [4] revealed that the two proteins had 37% identity and 56% similarity (data not shown). This finding may support those of previous studies [5, 8, 9].

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