Characterization and Structure of Alternatively Spliced Transcript Variant of Human Intestinal Alkaline Phosphatase (ALPI) Gene

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Summary     Intestinal-type alkaline phosphatase (IAP) is expressed at a high concentration in the brush border membrane of intestinal epithelial cells and is known to be a gut mucosal defense factor. In humans, a single gene (ALPI) for IAP has been isolated, and its transcription produces two kinds of alternatively spliced mRNAs (aAug10 and bAug10). Recently, we discovered that vitamin D up-regulated the expression of both types of human IAP alternative splicing variants in Caco-2 cells. However, the functional difference of protein encoded by the mRNA variants has remained elusive. In the present study, we aimed to provide further insight into the characterization and structure of IAP isoforms. To analyze the protein translated from the ALPI gene, we constructed two kinds of cDNA expression plasmids (aAug10 and bAug10), and the transfected cells were homogenized and assayed for alkaline phosphatase (ALP) activity. We also designed the homology-modeled 3D structures of the protein encoded by the mRNA variants (ALPI-aAug10 and ALPI-bAug10). The levels of ALP activity of COS-1 cells transfected with the aAug10 plasmid were increased significantly, while cells transfected with the bAug10 plasmid had undetectable ALP activity. The homology-modeled 3D structures revealed that the variant bAug10 lacks the central N-terminal α-helix and residue corresponding to Asp-42 of ALPI-aAug10 near the active site. This is the first report on the characterization and structure of alternatively spliced transcript variants of the human ALPI gene. Further studies on the regulation of aAug10 and/or bAug10 mRNA expression may identify novel physiological functions of IAP.

Key Words  human intestinal-type alkaline phosphatase, alternatively spliced mRNA variant, COS-1 cells, expression plasmid, homology-modeled 3D structure

Alkaline phosphatase (ALP: orthophosphoric monoester phospho-hydrolase, alkaline optimum, EC 3.1.3.1) is distributed widely throughout the living world from bacteria to animals, and it exists in various tissues such as bone, the liver, kidney, intestine, and placenta. ALP is an enzyme containing zinc which hydrolyzes monophosphate esters into inorganic phosphoric acid and alcohol at a high optimal pH (pH 8–10). In humans, there are primarily four different types of this enzyme: tissue-nonspecific ALP (liver/bone/kidney: TNSALP), intestinal-type ALP (IAP), placental-type ALP (PLAP), and germ cell ALP (1–4). Based on studies on cDNA encoding ALP isozymes, the primary structure in the catalytic region was demonstrated to be well-conserved in the ALPs of humans, animals, and Escherichia coli, suggesting that TNSALP, IAP, and PLAP play important roles in active metabolism by hydrolyzing phospho-compounds (1).

IAP is expressed at a high concentration in the brush border membrane of intestinal epithelial cells. IAP expression is lost with starvation, but refeeding stimulates IAP expression in mice (5). Recently, it was suggested that IAP may affect not only phosphate metabolism but also lipid metabolism based on an experiment using intestinal ALP knockout mice (6, 7). On long-term feeding of a high-fat diet, IAP knockout mice exhibited faster body weight gain than wild-type mice (6). However, in the IAP knockout mice, even those on a low-fat diet, glucose intolerance and the accumulation of visceral fat were observed, demonstrating characteristics of metabolic syndrome (7). Furthermore, the gram-negative bacterial cell wall component lipopoly-
saccharide (LPS) is known as an endotoxin; as IAP has the ability to detoxify LPS, IAP is a gut mucosal defense factor (5). ALPI mutation impaired the stability and catalytic activity of ALPI and led to the inability to detoxify LPS-dependent signaling, suggesting that ALPI deficiency may cause inflammatory bowel disease (8).

Although most species express a single IAP, several kinds of IAP have been identified in three species: the mouse (9), rat (10, 11), and cow (12). In rats, two different cDNA clones, IAP-I and IAP-II, for rat intestinal ALP were isolated by Lowe et al. (10) and Strom et al. (11), respectively. The two isozymes are products of two distinct genes and their cDNA sequences show 79% homology at the amino acid level. Functional differences between IAP-I and IAP-II were suggested by different regulations of the expression of the two mRNAs (13), as well as by structural and catalytic differences (14).

In humans, a single gene for human IAP has been isolated (ALPI gene), and the multiple forms of mRNAs encoding human IAP are due to differences in polyadenylation (2). The human ALPI gene (NCBI, GenBank Accession No. J03930) is located on chromosome 2 (2), and it contains 10 distinct introns. Recently, it was reported that transcription produces two kinds of alternatively spliced mRNAs [aAug10 (NM_001631) and bAug10 (M31008)] (NCBI, AceView) from the human ALPI gene (https://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?db=human&term=ALPI&submit=Go). The genomic organization and structure of alternatively spliced transcripts of human IAP are demonstrated in Fig. 1. The main variant aAug10 mRNA is
2,550 bp long (Fig. 1A), and it has been isolated from the small intestine, thalamus, rectum, ilea mucosa, and other tissues. The alternative variant bAug10 mRNA is 1,884 bp long (Fig. 1B), and it has been obtained from the kidney and thalamus. The two spliced mRNAs encode proteins (528 and 439 amino acids), comprising two different isoforms, containing the ALP domain and some transmembrane domains. Variant bAug10 contains a 150-bp-long upstream open reading frame (uORF) (Fig. 1B), and the efficacy of protein translation may be controlled by the presence of a shorter translated product (uORF) initiating at an AUG upstream of the main open reading frame (https://www.ncbi.nlm.nih.gov/IEB/Research/Assembly/a.cgi?db=human&term=ALPI&submit=Go).

Intestinal ALP is known to be affected by several kinds of nutrients (15-20). The level of ALP activity, a differentiation marker for intestinal epithelial cells, is regulated by vitamin D. Previously, we investigated the influence of vitamin D on the expression of two kinds of alternative mRNA variants encoding the human ALPI gene in human Caco-2 cells as an in vitro model of the small intestinal epithelium (20). After treatment with 1-alpha, 25-dihydroxyvitamin D3 [1,25(OH)2D3], the biologically active form of vitamin D3, there were significant increases in the ALP activities of Caco-2 cells. Reverse transcription-polymerase chain reaction analysis revealed that expression of both kinds of alternative mRNA variants from the ALPI gene was markedly enhanced by vitamin D in Caco-2 cells (20). However, the functional difference of protein encoded by the mRNA variants has remained elusive. To analyze the protein translated from the ALPI gene, we constructed two kinds of cDNA expression plasmids and homology-modeled 3D structures of human IAP isoforms (ALPI-aAug10 and ALPI-bAug10).

**MATERIALS AND METHODS**

Construction and analysis of the aAug10 or bAug10 cDNA expression plasmid. A full-length complementary DNA (cDNA) clone purified plasmid of human intestinal alkaline phosphatase was obtained from DNAFORM (Clone ID: 40146700, Kanagawa, Japan). After digestion of the plasmid vector DNA with EcoRI, the fragment containing human ALPI cDNA (aAug10) was isolated and inserted into the pcDNA3 expression vector (Invitrogen, Carlsbad, CA, USA) (Fig. 1A; aAug10 expression plasmid).

Complementary DNA synthesized from human adult small intestine total RNA (Takara Bio USA, Inc., Mountain View, CA, USA) was used to amplify the human ALPI cDNA (bAug10) was isolated and inserted into the pcDNA3 expression vector (Invitrogen, Carlsbad, CA, USA) (Fig. 1A; bAug10 expression plasmid).

Cell culture and transfection with aAug10 or bAug10 cDNA expression plasmid. COS-1 cells were grown at 37°C in 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, BRL) and antibiotics (100 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL of neomycin) (Gibco, BRL).

COS-1 cells were plated at a density of 1×10⁴ cells/cm² onto a 35-mm dish (FALCON, Bedford, MA, USA). Cells were transfected with circular plasmid DNA.
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(1 μg/35-mm diameter dish) by a Lipofectamine™ Reagent and Plus™ Reagent (Invitrogen). After 72-h transfection, cells were collected and homogenized using a Polytron homogenizer (Kinematica, Luzern, Switzerland) with 10 mM Tris-buffered saline (TBS) containing 1% Triton X-100 (pH 7.3) and 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 7,000 ×g for 5 min, the supernatant was used as the enzyme extract.

Enzyme activity and protein assays. The ALP activity was determined with 10 mM p-nitro-phenylphosphate (p-NPP) as a substrate in 100 mM 2-amino-2-methyl-1,3-propandiole HCl buffer containing 5 mM MgCl₂, pH 10.0, at 37°C, as previously reported (15). The enzyme activity was also assayed in the presence of an inhibitor: levamisole or L-phenylalanine (15). For thermostability, the enzyme preparation was pretreated at 60°C for 5 min and then reacted with the substrate (15). The enzyme activity was determined as the rate of hydrolysis of p-NPP and expressed in units (U=μmol p-nitro-phenol formed per minute).

Protein concentrations were determined using the bichinonic acid (BCA) protein assay reagent (Pierce, Rockford, IL, USA) (21).

Enzyme histochemistry. COS-1 cells on coverslips were stained for ALP activity. Cells were fixed with 10% formalin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) for 10 min on ice and washed three times with 0.1 M TBS. The cells were incubated with a mixture of 0.1 mg/mL of naphthol AS-MX phosphate (Sigma-Aldrich, Steinheim, Germany) and 0.4 M Tris-HCl buffer containing 5 mM MgCl₂ at room temperature for 30 min. Then, cells were counterstained with Fast red violet salt (Sigma-Aldrich) (22).

SDS-PAGE. Polyacrylamide gel (7.5%) electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed according to the method of Weber and Osborn (23). After electrophoresis, ALP isozymes separated in the gel were stained by the method of coupling β-naphthyl phosphoric acid monosodium salt with Fast blue B salt in 0.1 M 2-amino-2-methyl-1,3-propandiol-HCl buffer, pH 10.0 containing 1 mM MgCl₂ (22).

Reverse transcription-polymerase chain reaction (RT-PCR) analyses. Total RNA from the cells was extracted by the thioacrylamide-phenol-chloroform extraction method (CS104 RNAzol™ B, Tel-Test, Inc., Friendswood, TX, USA) (24). As a template for PCR, single-strand cDNA was prepared from 1 μg of total RNA using PrimeScript™ II 1st strand cDNA synthesis Kit (Takara Bio USA, Inc).

Fig. 3. (A) RT-PCR-based detection of mRNAs for variants aAug10 or bAug10 in the human adult small intestine in transfected COS-1 cells using specific primers. The PCR products (hIAP-a (480 bp) for aAug10 and hIAP-b (407 bp) for bAug10) were electrophoresed in a 5.25% polyacrylamide gel. Lane 1, the PCR products (hIAP-a or hIAP-b) from the human small intestine using the specific primers; lane 2, the PCR products (hIAP-a or hIAP-b) from COS-1 cells transfected with the Mock plasmid; lane 3, the PCR products (hIAP-a or hIAP-b) from COS-1 cells transfected with the aAug10 plasmid; lane 4, the PCR products (hIAP-a or hIAP-b) from COS-1 cells transfected with the bAug10 plasmid. GAPDH: glyceraldehyde-3-phosphate dehydrogenase. (B) The nucleotide sequence of the PCR product (hIAP-a) from COS-1 cells transfected with the aAug10 plasmid was compared with the human ALPI mRNA sequence [NCBI GenBank Accession No. NM_001631 (top sequence)]. The nucleotide sequences of the primers are underlined. Matches between the two sequences are marked by asterisks. The G in the GTC of the Val (valine)-1 codon of the mature form of variant aAug10 is denoted as nucleotide +1.
PCR primers were used for specific PCR primers: human IAP-a (hIAP-a) for the aAug10 mRNA variant (forward: 5′-ACCTTTCACCTCTTAGAGATA-3′; reverse: 5′-AGCAGGAAGCTTGCAAGAAG-3′), and human IAP-b (hIAP-b) for the bAug10 mRNA variant (forward: 5′-GCTGACGTAGCTCTACTCTT-3′; reverse: 5′-ACCTCA-TTGCGGCGTGTCGT-3′) (20).

The PCR was performed using simpliAmp Thermal Cycler (Applied Biosystems). The amplified samples were analyzed using 5.25% polyacrylamide gel electrophoresis (PAGE). The stained gels were observed with UV light.

Statistical analyses. Values are shown as the mean ± standard error (S.E.) from triplicate experiments. The Tukey test was used after one-way ANOVA to compare the significance of differences in ALP activities and protein concentrations among all groups. Differences were considered significant at p < 0.05. Analysis was conducted using IBM SPSS Statistics (version 22, IBM Corporation, Somers, NY, USA).

3D structures of human ALPI-aAug10 and ALPI-bAug10. Amino acid sequence alignment of human placental ALP (PLAP) (25) and IAPs (ALPI-aAug10 and ALPI-bAug10) is shown in Fig. 2. The homology-modeled 3D structures of human ALPI-aAug10 and ALPI-bAug10 were constructed using Discovery Studio v. 4.1 (Accelrys, San Diego, CA, USA) based on the crystal structure of human PLAP (PDB:3MK1) (26).

RESULTS

Detection of human ALPI splicing variants

In order to verify the expression of aAug10 or bAug10 mRNA, we performed RT-PCR analysis using specific primers. The PCR products [hIAP-a (480 bp) for the aAug10 and hIAP-b (407 bp) for the bAug10] were electrophoresed in a 5.25% polyacrylamide gel. As shown in Fig. 3A (lane 1), we confirmed both the expression of aAug10 and bAug10 mRNAs in human adult small intestine total RNA as a positive control. We detected hIAP-a from COS-1 cells transfected with the aAug10 plasmid [Fig. 3A (lane 3)] and hIAP-b from COS-1 cells transfected with the bAug10 plasmid [Fig. 3A (lane 4)].

The nucleotide sequences of the PCR products (hIAP-a and hIAP-b) from COS-1 cells transfected with the aAug10 or bAug10 plasmid were identical to the human ALPI sequence [NCBI GenBank, Accession No. NM_001631, M31008] (Fig. 3B and 3C).

Expression of the ALP activity and enzyme histochemistry

To examine the effect of the alternatively spliced transcript variant on the catalytic properties of COS-1 cells transiently expressing the human ALPI gene, the cells were homogenized and assayed for ALP activity. As shown in Fig. 4A, COS-1 cells transfected with aAug10 plasmid expressed significantly higher levels of ALP activity than COS-1 cells transfected with bAug10 plasmid had undetectable ALP activity.

The results of the inhibition and thermal inactivation experiments in the enzyme preparations of COS-1 cells are shown in Table 1. Remaining ALP activity following the treatments is expressed as a percentage of the untreated control. The enzyme preparations of COS-1 cells transfected with aAug10 plasmid were effectively inhibited by L-phenylalanine but not by levamisole, and were heat-stable (60˚C, 5 min), corresponding to the property of other mammalian intestinal-type ALPs (27).

As shown in Fig. 4B, cytohistochemical staining confirmed the presence of strong ALP activity at the surface of COS-1 cells transfected with the aAug10 plasmid, while COS-1 cells transfected with the bAug10 plasmid had undetectable ALP activity.

SDS-PAGE analysis

The further identification of enzymatic properties of intestinal-type ALP isozymes was performed using SDS-PAGE (Fig. 4C). In the enzyme preparations of COS-1 cells transfected with the aAug10 plasmid, we detected a main band of ALP activity, and the molecular weight was 110 kDa as calculated from the positions of the standard proteins [Fig. 4C (lane 2)]. However, no band of ALP activity in COS-1 cells transfected with the bAug10 plasmid was detected [Fig. 4C (lane 3)].

The homology-modeled 3D structure of human ALPI

To compare the structural differences in the protein translated from the ALPI gene, we constructed a homodimeric model of IAPs (ALPI-aAug10 and ALPI-bAug10) based on the crystal structure of human PLAP (26). As shown in Fig. 5A, the overall structure of human IAP (ALPI-aAug10) is a dimer; each monomer contains 528 amino acids and the residues involved in the active site of the enzyme [Ser (serine)-92], and ligands coordinating the three metal ion sites [two zinc (Zn1 and Zn2) and one magnesium] are conserved (27). Then, we demonstrated the overall structure of ALPI-bAug10 as a dimer; each monomer contains 439 amino acids and lacks 70 amino acids on the 5′ terminal side (Fig. 5B). The residue involved in the putative active site of the enzyme (ALPI-bAug10) is suggested to be Ser-22 corresponding to Ser-92 of ALPI-aAug10. The modeled 3D structures revealed that ALPI-bAug10 lacks the central N-terminal long α-helix of ALPI-aAug10, highlighted in yellow in Fig. 5C. Figure 5D and 5E show the comparison of the active site region in the 3D modeled structure of ALPI-aAug10 (Fig. 5A) or ALPI-bAug10 (Fig. 5B). The expected phosphate-binding residue (Ser-92 or Ser-22) coordinating the chelating zinc atom (Zn) was observed near the active site in the modeled 3D structure of both ALPI-aAug10 and ALPI-bAug10 (Fig. 5D and 5E). However, the residue corresponding to Asp (aspartic acid)-42 of ALPI-aAug10 near Zn was not recognized in ALPI-bAug10 because of the deletion of 70 amino acids on the 5′ terminal side (Fig. 5E).

DISCUSSION

This study investigated the characterization and structure of alternatively spliced transcript variants of the human ALPI gene. To analyze the protein translated from the ALPI gene, we constructed two kinds of cDNA expression plasmids (aAug10 and bAug10). As COS-1 cells did not express a significant amount of endoge-
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In our previous publication (28), we examined the catalytic properties of ALP in COS-1 cells transiently expressing the human ALPI gene. COS-1 cells transfected with the aAug10 plasmid expressed significantly higher levels of ALP activity, while COS-1 cells transfected with the bAug10 plasmid had undetectable ALP activity.

ALP activities of COS-1 cells transfected with the aAug10 plasmid were effectively inhibited by L-phenylalanine but not by levamisole and were heat-stable. Previously, we reported that human intestinal ALP showed heat stability, but TNSALPs in human bone, liver, kidney, and dental tissues lost >50% of their activity within 10 min at 56°C (29). Levamisole is an effective inhibitor of TNSALP, but it is not effective against IAP. On the other hand, L-phenylalanine is an effective inhibitor of IAP, but is not effective against TNSALP. These biochemical properties of the enzyme preparations of COS-1 cells transfected with the aAug10 plasmid were identical to human intestinal ALP.

Cyt histochemical staining demonstrated the presence of strong ALP activity at the surface of COS-1 cells transfected with the aAug10 plasmid, while COS-1 cells transfected with the bAug10 plasmid had undetectable ALP activity. The protein encoded by bAug10 may not be secreted extracellularly due to the lack of the signal peptide; thus, the surface of COS-1 cells transfected

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<th>Table 1. Biochemical properties of alkaline phosphatase in COS-1 cells transfected with the aAug10 expression plasmid</th>
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<td>Remaining ALP activity (%)</td>
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<td>Levamisole (5 mM)</td>
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<td>aAug10</td>
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ALP activity was assayed by the rate of hydrolysis of p-nitrophenylphosphate. The effects of the inhibitor were determined in the presence of 5 mM MgCl₂ in the assay mixture. Activities of non-treated controls were designated as 100%. Cultured cells were transfected with the aAug10 plasmid for 72 h. Each value represents the mean±S.E. (n=3) of triplicate experiments.
with bAug10 plasmid was not stained for ALP activity.

By SDS-PAGE analysis, COS-1 cells transfected with the aAug10 plasmid were also found to express high levels of ALP activity, and the molecular weight of ALP-aAug10 under nonreducing conditions was estimated to be about 110 kDa, which is consistent with the dimeric form of native mammalian ALPs.

In order to examine the reason why ALP-bAug10 had undetectable ALP activity, we compared the 3D structures between ALP-aAug10 and ALP-bAug10. The overall structure of ALP is a dimer structure, and it is considered that the interaction between subunits causes an allosteric effect and regulates ALP enzyme activity and reaction to inhibitors (27, 30). Hoylaerts et al. reported that mammalian ALPs are zinc-containing metalloenzymes and functional as dimeric molecules,
but when separated the monomeric subunits fail to display enzyme activity (30).

*Escherichia coli* ALP is one of the most well-studied enzymes both biochemically and structurally (31). The surface of PLAP is conserved with that of *Escherichia coli* ALP with only 8% residues in common, and additional secondary structure elements, including an N-terminal α-helix (resides 9–25), an α-helix and a β-strand in a highly divergent region (resides 208–280), and a different organization of the small β-sheet in domain 365–430 are observed in human PLAP (27). The human PLAP molecule is known to show 87% homology with IAP (ALPI-aAug10) at the amino acid level (32), and all these structural features discovered in PLAP are conserved in IAP (ALPI-aAug10), with no insertion or deletion relative to PLAP. The modeled 3D structures revealed that ALPI-bAug10 lacks the central N-terminal long α-helix of ALPI-aAug10. This N-terminal long α-helix has been shown to play an important role in defining the conformation and stability of the ALP molecule (27, 30).

Furthermore, the residues that are essential for catalysis are preserved in the active site of *Escherichia coli* ALP, that is the catalytic Ser and the three metal ion sites [two zinc, Zn1 and Zn2], and one magnesium (27). We compared the active site regions in the homology-modeled 3D structures of ALPI-aAug10 and ALPI-bAug10. Each expected phosphate-binding residue (Ser-92 or Ser-22) coordinating the chelating zinc atom (Zn) was observed near the active site in the modeled 3D structure of both ALPI-aAug10 and ALPI-bAug10. However, in ALPI-bAug10, the residue corresponding to Asp-42 of ALPI-aAug10 near Zn was not recognized because of the deletion of 70 amino acids on the 5′ terminal side. Asp-42 is related in chelating the zinc atom in ALPI-aAug10; therefore, we considered that ALPI-bAug10 showed undetectable ALP activity due to the lack of the residue corresponding to Asp-42 of ALPI-aAug10. Recently, the crystal structure of rat IAP was reported by Ghosh et al. (33). They compared the structure of human PLAP with rat IAP, and demonstrated that all the residues in direct contact with the metal ions are conserved. In the active site region, the residues of Asp-42, Asp-316, His (histidine)-320, His-432, and Ser-92 of ALPI-aAug10 are conserved in the crystal structure of rat IAP. Asp-316, His-320, and His-432 coordinating the chelating Zn1, and Zn2 stabilized by interaction with Asp-42 and Asp-316.

The physiological role of IAP still remains unclear, but may have multiple biological roles in the maintenance of intestinal homeostasis and diet modulation. It is well-known that IAP is a marker of intestinal differentiation, and high-level activity of the intestinal ALP enzyme is considered to be involved in intestinal differentiation. Previously, we demonstrated that the ALP activities of human Caco-2 cells treated with 1,25(OH)2D3 (100 nM) were significantly higher in the 1,25(OH)2D3-treated group compared with control group on day 3 (20). Then, on days 5 and 7, intensities of aAug10 expression in Caco-2 cells were not significantly higher in the 1,25(OH)2D3-treated group compared with control group, while the intensities of bAug10 expression in Caco-2 cells were significantly higher in the 1,25(OH)2D3-treated group compared with control group (20). These different time-course gene expression profiles of variants aAug10 and bAug10 suggested the importance of the transcriptional regulation of human ALPI gene expression. However, in the present study, the transcriptional regulation of aAug10 and/or bAug10 mRNA expression has remained elusive.

Comparing with aAug10 and bAug10 mRNAs, the mRNA size. 5′ untranslated region, and 3′ untranslated region are different. That is, the size of bAug10 mRNA is 1,884 bp and it is smaller than aAug10 mRNA (2,550 bp) because the 3′ untranslated region of exon11 in bAug10 is shorter than that of aAug10 mRNA. However, the 3′ untranslated region of bAug10 mRNA does not affect C-terminal amino acid sequences. The 5′ untranslated region of bAug10 mRNA contains a 150-bp-long upstream open reading frame (uORF) that controls initiation at an AUG. As the results, 70 amino acids in the N-terminal region are deleted in variant bAug10. The 70 deleted amino acids include a central N-terminal long α-helix for the conformation and stability of the ALP molecule, the residue corresponding to Asp-42 of ALPI-aAug10 for the chelating Zn, and the signal peptide region for extracellular secretion.

The present study demonstrated the mRNA expression of aAug10 and bAug10 with commercially available total RNA of the human small intestine. In the future, various types of human small intestinal tissues (duodenum, jejunum, and ileum) should be sampled to quantify the mRNA tissue expression of aAug10 and bAug10. Moreover, bAug10 may have effects, such as inflammation and biological defense, in addition to ALP activity, and regulate ALP activity due to aAug10. Therefore, in the future, anti-aAug10 and -bAug10 antibodies should be prepared to examine protein expression levels.

In conclusion, we demonstrated that ALPI-bAug10 had undetectable ALP activity on the deletion of 70 amino acids on the 5′ terminal side. Although the phosphate-binding residue corresponding to Ser-92 of ALPI-aAug10 was observed in ALPI-bAug10, the residue corresponding to Asp-42 of ALPI-aAug10 that is involved in chelating the zinc atom near the active site was not recognized in ALPI-bAug10. Further studies on the physiological functions of human IAP (ALPI-aAug10 and ALPI-bAug10), the transcriptional regulation of IAP induction, and relationship with nutritional factors will provide useful data on mucosal protective factors for the prevention of inflammatory bowel diseases.

**Authorship**

SN and MG carried out molecular genetic studies and
developed the study concept and design. SN, AY, and MG carried out the analysis and interpretation of data and drafting of the manuscript. YA and HN developed the homology-modeled 3D structure and conducted critical revision of the manuscript for important intellectual content. TM and HO provided the technical support and study supervision. All authors read and approved the final manuscript.

Disclosure of state of COI
The authors disclose no conflicts.

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REFERENCES


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