Heat shock induces intestinal-type alkaline phosphatase in rat IEC-18 cells

TSUYOSHI HARADA,1 IWAO KOYAMA,1 TOSHIHIKO KASAHARA,1 DAVID H. ALPERS,2 AND TSUGIKAZU KOMODA1
1Department of Biochemistry, Saitama Medical School, Saitama 350-0495, Japan; 2Division of Gastroenterology, Washington University School of Medicine, St. Louis, Missouri 63110
Submitted 24 June 2002; accepted in final form 30 September 2002

Harada, Tsuyoshi, Iwao Koyama, Toshihiko Kasahara, David H. Alpers, and Tsugikazu Komoda. Heat shock induces intestinal-type alkaline phosphatase in rat IEC-18 cells. Am J Physiol Gastrointest Liver Physiol 284: G255–G262, 2003. First published October 23, 2002; 10.1152/ajpgi.00244.2002.—We demonstrate a previously unknown regulation for intestinal-type alkaline phosphatase (IAP) as a heat shock protein (HSP). Heat shock to rat intestinal epithelial cells (IEC-18) at 43°C induced the expression of IAP-I and HSP72 mRNAs time dependently (<60 min) but did not induce expression of IAP-II, tissue nonspecific-type alkaline phosphatase (TNAP), or HSP90 as determined by the RT-PCR method. To confirm the identity of the IAP-I gene, we sequenced the amplification product of IAP-I and found the gene to have 99% homology with the sequence of the IAP-I gene in rat intestine. Under the subculture conditions used, no IAP protein was detected in IEC-18 cells, but it became detectable as a 62-kDa band on a Western blot after heat shock. IAP-I was also induced by sodium arsenite, which generates reactive oxygen species and is an inducer of members of the HSP family. Glutathione suppressed activating protein-1 and cAMP response element-binding protein activation caused by heat shock but did not suppress the expression of IAP-I. These results suggest that cellular stress induces the elevation of IAP-I mRNA and protein synthesis. IAP-I may play an important role as a dephosphorylating enzyme under stress conditions.

ALKALINE PHOSPHATASE (AP; EC 3.1.3.1) is an ectoenzyme anchored to the membrane by a glycan-phosphatidylinositol (GPI) anchor moiety and hydrolyzes a variety of monophosphate esters at alkaline pH (35). In primates, the AP gene family consists of four distinct loci types, i.e., the tissue nonspecific AP (TNAP), which is expressed mainly in liver, bone, and kidney; the intestinal AP (IAP); the placental AP (PLAP); and the germ cell AP (GCAP) (11). It has been proposed that, during the evolution of the AP gene family, the first duplication from an ancestral AP gene produced TNAP, and subsequent duplications gave rise to further modifications resulting in the IAP, PLAP, and GCAP genes (21, 26). TNAP has been shown to be heat labile and IAP, PLAP, and GCAP to be heat stable, with IAP resisting temperatures ≈56°C and PLAP and GCAP resisting temperatures ≈70°C (12, 28). On the basis of these findings, we hypothesized that the evolution of AP isozymes has been associated with the acquisition of heat resistance; however, the physiological function(s) and substrate(s) of heat-stable AP isozymes and the significance of their heat-resistant nature remain uncertain.

LPS, which is a pyrogen, has recently been proposed as a candidate substrate of AP, and two phosphate groups of lipid A, its toxic core, have been found to be dephosphorylated by AP at physiological pH (43, 44). LPS has been shown to induce AP in the duodenum, lungs, and liver and in a small intestinal epithelial cell (IEC) line (16, 44, 49); and an increase in the levels of intracellular cAMP, mediated by inflammatory factors, such as cytokines and activation of protein kinase A, has been shown to be involved in the regulation of AP expression (4, 23, 27, 41). However, little is known about the effect of fever itself on the expression of heat-stable AP.

Because no PLAP and GCAP isozymes have ever been detected in rat tissues, IAP is the most heat-resistant isozyme known in rats. IAP is mainly distributed in the intestines, but exigous synthesis has been observed in kidney, liver, and lung tissue (14, 18). Rat intestine produces two distinct isozymes of IAP, IAP-I, and IAP-II, which have 79% amino acid identity and differ markedly at their COOH-terminal end (10, 47, 53). These isozymes appear at different times during postnatal development, have different substrate specificities, and respond differentially to cortisone or cortisone plus thyroxine, 1,25-dihydroxyvitamin D3, fat feeding, and LPS (47, 49, 55).

All organisms respond to heat by inducing the rapid synthesis of heat shock proteins (HSPs). Response is the most highly conserved genetic system known, existing in every organism in which it has been sought, from archaeabacteria to eubacteria, from plants to animals. Among rat IEC lines, the heat shock response of HSP has been studied in IEC-18 cells derived from the...
ileum of a rat in the suckling stage (2, 40, 50, 52). Despite the large amount of information on the genetic regulation of HSPs and their role as chaperones, little is known about the regulation and role of AP isozymes in relationship to the heat shock response. In the present study, we used nontransformed IEC-18 cells to investigate whether nonlethal thermal stress influences the expression of AP isozymes in the cells.

MATERIALS AND METHODS

Cell culture and heat/chemical shock treatment. IEC-18 cells (passage 30–34) were routinely cultured in plastic culture flasks containing DMEM supplemented with 10% fetal calf serum, 10 mM glutamine, 50 U/ml penicillin, and 50 
μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO2-95% air. Cells were subcultured weekly by using 0.05% trypsin-0.02% EDTA in PBS without Ca2+ and Mg2+.

Once the cells became confluent in the flasks (25 cm2 in size), lids of the flasks were quickly fastened tightly to avoid any loss of CO2 just before heat shock. To induce HSPs, we subjected IEC-18 monolayers to the nonlethal temperature of 43°C in a water bath for various lengths of time. At the completion of heat shock, total RNA was immediately extracted from the cells, or the flasks were lifted from the water bath, their lids were loosened, and they were promptly returned to the incubator at 37°C for a predetermined time.

Some IEC-18 cells were pretreated with DMEM containing 10 
μg/ml actinomycin D for 2 h, 200 
μg/ml sodium arsenite for 1 h, or 30 mM glutathione (GSH) for 1 h. Before this preincubation, the medium was replaced with fresh DMEM, and the cells were subjected to the heat shock procedure described above.

RNA preparation and PCR. Total cellular RNA was isolated with a commercial kit (Isogen; Nippon Gene, Tokyo, Japan) according to the protocol provided by the manufacturer. cDNAs were reverse transcribed from total RNA (4 
μg) with a Qiagen Omniscript reverse transcriptase kit by using the oligo(dT)15 primer (Roche, Mannheim, Germany). PCR amplification of the IAP-I, IAP-II, TNAP, HSP72, and HSP90 transcripts was performed with a KOD-Fla polymerase kit (Toyobo, Osaka, Japan). Sequences of the PCR primers for these transcripts were derived from the following published sequences: IAP-I, sense 5′-CCCCCGAGGGCAAGAACCCATACACCGACT-3′ and antisense 5′-GGCCACCGTGGAGACCCTGGC-3′ designed to amplify a fragment corresponding to nucleotides 4487–4768, (53); IAP-II, sense 5′-CCTGGAGCCCTACACCGACT-3′ and antisense 5′-GCCACCGTGGAGACCCTGGC-3′ for nucleotides 4221–4476, (53); TNAP, sense 5′-AGTCCGGT- GGGCATCGTGAC-3′ and antisense 5′-GTGGAGGTCCGT- GTGTCCTAG-3′ for nucleotides 477–803, (36); HSP72, sense 5′-TCGAGGAGGTGGATTAGAG-3′ and antisense 5′-GGTGGAGGTCCGT- GTGTCCTAG-3′ for nucleotides 477–803, (36); HSP72, sense 5′-TCGAGGAGGTGGATTAGAG-3′ and antisense 5′-GGTGGAGGTCCGT- GTGTCCTAG-3′ for nucleotides 477–803, (36); and HSP90, sense 5′-ACATCTCCCCCAACCTC-3′ and antisense 5′-CTCAGCAGAAGACTCC-3′ (1). PCR primers for rat β-actin were purchased from Clontech Laboratories (Palo Alto, CA). The optimum number of cycles for the PCR of each primer pair was determined by serial dilutions of the cDNA in the PCR reactions until a linear response was obtained. The optimum number of cycles for each primer pair was: 35 for IAP-I, 40 for IAP-II, 34 for TNAP, 28 for HSP72, 23 for HSP90, and 23 for β-actin. PCR parameters for all cDNAs were denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 45 s. The PCR products were separated by electrophoresis on 2% agarose gels. DNA was visualized by ethidium bromide staining. Intensity of the bands was evaluated with a charge-coupled device camera system (Atto, Tokyo, Japan).

Sequencing. Cycle sequencing was performed on a GeneAmp PCR System 9600 (PerkinElmer, Norwalk, CT) utilizing a PRISM Ready Dye Terminator Cycle Sequencing kit with AmpliTaq DNA polymerase, FS [Taq-FS; PerkinElmer/Applied Biosystems Division, Foster City, CA], according to the manufacturer’s recommendations. Briefly, the gel-purified DNA (10.4 
μl) was added to a MicroAmp reaction tube (PerkinElmer) containing 3.2 pmol of sequencing primer and 8.0 
μl of premix [containing buffer, 2-deoxynucleotide 5′-triphosphates (dNTPs), dye-labeled ddNTPs, and Taq-FS/pyrophosphatase]. After the initial denaturation at 96°C for 2 min, the reaction mixture was incubated for 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Excess dye-labeled terminators were removed from the extension products by spin-column purification (CentriSep spin column; Princeton Separations, Adelphia, NJ) according to the manufacturer’s directions. Once separated, the extension products were evaporated to dryness under reduced pressure (SpeedVac; Savant Instruments, Farmingdale, NY). Each sample was then resuspended in 2 
μl of sequencing buffer (5.1, 1% deionized formamide/50 mM EDTA, pH 8.0), heated for 2 min at 90°C, and loaded as a 0.5 
μl aliquot onto an Applied Biosystems PRISM 377XL sequencer.

Western blotting. After heat shock for 60 min, IEC-18 cells were cultured at 37°C for 3 h, washed 2 times with PBS, and then scraped with a rubber policeman into 1 ml of lysis buffer (10 mM Tris-HCl buffer, pH 7.8, containing 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine chloride). An equal volume of n-butanol was added to the collected cell lysates, and the mixture was stirred at room temperature for 15 min and then centrifuged at 4,000 
×g for 30 min. The aqueous phase was collected, cold acetone (−20°C) was added to a final concentration of 60% (vol/vol), and the solution was stored at −20°C overnight. Precipitate was collected by centrifugation and dried to an acetone powder. The acetone powder was solubilized with sample buffer (60 mM Tris-HCl buffer, pH 6.8, containing 1% SDS, 10% glycerol, and 5% 2-mercaptoethanol) and boiled for 5 min. These samples were subjected to electrophoresis on a SDS-PAGE (8% acrylamide) gel under reducing conditions. Separated proteins were transferred to immobil-P membranes (Millipore, Bedford, MA) at 0.4 mA for 1 h at 4°C and blocked overnight in Tris-HCl-buffered saline, pH 7.8, containing 5% nonfat dry milk and 0.1% Tween 20. Membranes were then washed with Tris-HCl-buffered saline plus 0.1% Tween, and IAP bands were detected by using the rabbit anti-rat IAP antiserum characterized previously (54). The membranes were incubated for 1 h at room temperature with the primary antibody at a 1:5,000 dilution in the buffer. After being washed with Tris-HCl-buffered saline plus 0.1% Tween, the membranes were incubated for 1 h at room temperature with anti-rabbit horseradish peroxidase-linked antibody at a 1:10,000 dilution as the secondary antibody. IAP bands detected by the antibodies were visualized by using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Little Chalfont, UK).

Preparation of nuclear extracts and transcription factor activation assay. Cells were harvested by removing the incubation medium, rinsing the cells twice with 10 mM PBS, pH 7.5 containing 150 mM NaCl, 2.7 mM KCl, and 10 mM of a cocktail of phosphatase inhibitors [in (mM) 5 NaF, 10 β-glycerophosphate, 10 paranitrophenyl phosphate, 1 NaVO4], scraping the cells off the substratum with a rubber policeman, and pelleting them by centrifugation at 1,000 
×g for 5 min at 4°C. The pellet was then resuspended in 300 
μl of 20

AJP-Gastrointest Liver Physiol • VOL 284 • FEBRUARY 2003 • www.aajpgi.org
mM HEPES buffer, pH 7.5, containing (in mM) 350 NaCl, 1 MgCl₂, 0.5 EDTA, 0.1 EGTA, and 20% glycerol, 1% NP-40, and a protease inhibitor cocktail (Roche, Germany). After 10 min on ice, the lysate was centrifuged for 20 min at 100,000 g. The supernatant constituted the total protein extract and was kept frozen at −80°C. The protein concentration of the nuclear extracts was measured with a bicinchoninic acid kit (Pierce, Rockford, IL).

The activity of activator protein-1 (AP-1) and cAMP response element-binding protein (CREB) DNA-binding activities were determined with ELISA-based assay kits (TransAM) obtained from Active Motif (Carlsland, CA). In brief, the nuclear extracts were added to microwells coated with a cold oligonucleotide containing the consensus-binding site for activator protein-1 (AP-1) or CREB. After 1-h incubation at room temperature, the microwells were washed three times with washing solution. Antibodies directed against phosphorylated c-Jun or phosphorylated CREB were used to label the AP-1 or CREB dimers bound to the oligonucleotide and followed by a secondary antibody conjugated to horseradish peroxidase. Finally, the results were quantified by a chromogenic reaction (46).

RESULTS

Effect of heat shock on expression of the mRNA of AP isozymes and HSPs. To investigate whether exposure of IEC-18 cells to nonlethal heat would stimulate the expression of AP isozymes and, if so, to determine the optimal time for induction of their expression, we exposed the cells to a temperature of 43°C for various lengths of time from 0 to 60 min. RT-PCR analysis of IAP-I, IAP-II, TNAP, HSP72, HSP90, and β-actin expression was then performed. RT-PCR with both IAP-I and IAP-II primers before heat stimulation did not yield their amplification products, which were 282 and 256 bp, respectively (Fig. 1A). Amplification products of TNAP, HSP72, HSP90, and β-actin were observed as single bands of 327, 307, 269, and 764 bp, respectively, on gels loaded with samples from the untreated cells (37°C). The number of amplification cycles used for these genes was lower than that used for IAP-I or IAP-II (Fig. 1, A and B).

In the samples from the heat-shocked IEC-18 cells, the amplification products of IAP-I and HSP72 dramatically increased 60 min in a time-dependent manner during the observation period; but no band for IAP-II was detected at 256 bp in this experiment.

Cloning of the PCR product of IAP-I. The IAP-I PCR product was cloned by the direct sequence method (Fig. 2). The nucleotide sequence of the PCR product in this experiment had 99.0% homology with the sequence reported by Xie and Alpers (53). Three sites of single-nucleotide polymorphism were detected, which reflected the open reading frame of the IAP-I protein at nucleotide 4601, but the corresponding codon was the same degeneracy.

To reconfirm the induction of the IAP-I gene, we sequenced the PCR product amplified from the exon 8 region (nucleotide position: 3282–3671) (53) with another set of primers. This product was also induced by heat shock and was 99.9% homologous to the rat IAP-I gene (data not shown).

IAP-I gene transcription. As supportive evidence that the induction of IAP-I by thermal stress was mediated by transcriptional activation of the IAP-I gene, the cells were exposed to the RNA polymerase inhibitor actinomycin D (10 μg/ml). As shown in Fig. 3, the time-dependent increases in the expression of IAP-I...
and HSP72 were completely or partially inhibited by exposure to the inhibitor. These findings indicate that the increase in IAP-I or HSP72 expression was due to an increase in synthesis rather than to a decrease in the rate of IAP-I mRNA degradation.

Production of IAP protein by heat shock. To assess whether the IAP-I expression was also elevated at the translational level, we performed Western blot analysis with antiserum raised against specific IAP protein (Fig. 4), and the 62-kDa IAP band was detected in the blot of the gel lane containing a sample from the heat-shocked IEC-18 cells.

Effect of arsenite and GSH on expression of the IAP-I gene. Arsenite is known to be capable of inducing stress proteins, including HSP70, as well as inducing thermostolerance (32, 51). Exposure of IEC-18 cells to arsenite increased the levels of expression of the mRNAs of HSP72 and IAP-I but not of TNAP (Fig. 5). Expression of HSP72 and IAP-I mRNAs in the arsenite-exposed IEC-18 cells was greater than that in the heat shock-treated IEC-18 cells. In contrast to the nontreated cells, heat shock did not dramatically induce the expression of HSP72 and IAP-I in arsenite-pretreated cells. These results suggest that the regulation of IAP-I is related to oxidative stress, because arsenic generates reactive oxygen species.

It has been suggested that heat shock impairs the cellular redox balance and that AP-1-DNA binding activity is increased through activation of c-Jun after heat shock (8, 48). Because AP-1 and CREB/ATF-DNA binding motifs are present as enhancer elements within the sequenced 5'-flanking region of IAP-I (53), we then sought to determine whether activation of these transcriptional factors in response to heat shock is involved in the regulation of the heat-induced increase in IAP-I gene expression. Fig. 6A shows that, compared with their activity in nuclear extracts from uninduced cells, AP-1 and CREB-DNA binding activity was induced by heat shock as well as by arsenite exposure. Exposure of cells to 30 mM GSH for 60 min before heat shock reduced the heat shock induction of AP-1 and CREB binding activity to the control levels but not the arsenite-induced activity.

Having found that GSH prevented the heat shock-induced activation of AP-1 and CREB-DNA binding, we then examined the effect of GSH on the expression of IAP-I in heat-treated cells. Exposure of cells to GSH did not induce expression of IAP-I or HSP72 (Fig. 6B). After being heat shocked, cells were pretreated with GSH; however, the expression of IAP-I was increased to the level observed in the heat-shocked cells without GSH pretreatment.

DISCUSSION

The heat shock response is mediated by increased expression of genes encoding a group of proteins referred to as the HSP family or stress proteins (33, 38). HSPs are crucial for the maintenance of cell integrity during normal cell growth as well as under certain pathophysiological conditions, and they are thought to support the transportation, folding, and rearrangement of other proteins by acting as chaperones (9).
Members of the HSP family have been classified according to their apparent molecular weights, functions, and inducers, respectively. However, IAP has never been previously reported to be a heat shock responder. The present study demonstrates for the first time, to our knowledge, that heat treatment is capable of inducing IAP production in rat IECs.

Instead of being regulated by increased levels of a transcriptional activator, HSP gene transcription is regulated by the activation of a preexisting pool of heat shock transcription factors (HSF) that bind to the HSP promoter element (HSE; Ref. 38). For example, HSF1 is folded and maintained in a non-DNA-binding state as a monomer under normal physiological conditions, and activation of HSF1 is mediated by disruption of intramolecular interactions, which results in a homotrimeric form that binds to HSE (37). Xie and Alpers (53) sequenced the 5′-flanking region (~1.7 kbp) of the rat IAP-I gene but found no typical HSE within this region. Recently, several reports have indicated that HSP itself influences the expression of other genes. Exogenous HSP70 acts as a cytokine by stimulating a proinflammatory signal transduction cascade that results in an upregulation of IL-1β, IL-6, and tumor necrosis factor-α expression through both CD14-dependent and -independent pathways (3), and these cytokines or transcriptional factors activated by HSP70 may be associated with the expression of heat-induced IAP-I. In the present study, we confirmed that the response of the elevated level of IAP-I mRNA caused by heat shock was linked to that of HSP72 and was due to synthesis, because pretreatment with actinomycin D blocked the elevation, indicating transcriptional activation of the IAP-I and HSP72 genes. We also confirmed that arsenite has the ability to induce expression of IAP-I mRNA as well as HSP72 mRNA.

Arsenicals have been shown to generate reactive oxygen species and cause the induction of a number of major stress proteins (7). It has been suggested that heat shock impairs the cellular redox balance, resulting in generation of reactive oxygen species (6, 13). Alterations in intracellular oxidation/reduction reactions have been shown to activate signal transduction cascades that regulate early response genes (19, 25), and AP-1-DNA binding activity is also activated through the activation of these genes after heat shock (8, 48). Moreover, the enhancer motifs of AP-1 and CREB/ATF have been found within the sequenced 5′-flanking region of the rat IAP-I gene (53). However, we showed that heat treatment of IEC-18 cells after pretreatment with GSH at the relatively high concentration of 30 mM still induced expression of IAP but did not activate AP-1 and CREB/ATF DNA binding. These findings indicate that heat shock can induce the expression of IAP-I, even when reactive oxygen species-related signal pathways are suppressed. It is therefore likely that the regulatory mechanism of the heat shock response of the IAP-I gene involves the HSF-HSE system upstream of the certified 5′-flanking region. However, it is unclear at the present time whether the regulation of heat-induced IAP-I is mediated by some other transcription factor indirectly associated with HSF or by some other unknown mechanism.

It has been reported that TNAP is the predominant isozyme in IEC cell lines and can be induced by retinoic acid, 1,25-dihydroxycholecalciferol, and butyrate (15, 24, 42). Expression of IAP genes had not been detected previously in IEC cell lines, because the cell lines were derived from neonatal rat small intestines and retained features characteristic of immature, fetal-like crypt cells, as judged by immunologic and morphological criteria (24, 45). In the fetal rat intestine, TNAP is
expressed primarily in the single layer of cells lining the primitive gut during the first phase of gestation, and the cells lining the newly developed crypt cells during the second phase also express TNAP. However, TNAP expression changes to IAP expression during the third gestational phase (26). After the postnatal surge of IAP production, the intestinal epithelium becomes the tissue containing the largest amount of this enzyme, and this conversion completes the maturation by morphogenesis and function (56). IAP is a late evolutionary development in the AP gene family, having appeared first in mammals and before PLAP appeared in primates (17, 26). The emergence of IAP may be of significance in relationship to the hydrolysis and metabolism of phosphorylated substances during the development of rat intestine.

Two distinct IAP isoenzymes are expressed in the rat intestine and are encoded by different mRNAs. IAP-I is a 65-kDa AP isozyme in the rat intestine and is the product of a 2.7-kbp mRNA, whereas IAP-II is a 90-kDa AP isozyme encoded by a 3.0-kbp mRNA (53, 56). Regulatory differences among IAP isozymes have been demonstrated by the fact that IAP-II, but not IAP-I, is stimulated by fat feeding, cortisone, and 1,25-dihydroxycholecalciferol. On the other hand, IAP-I emerges earlier than IAP-II in the neonatal development of rat intestine (55), and its expression is stimulated by LPS-inoculation of rat lung (20). We confirmed that AP activity and 70-kDa IAP protein, probably originating from the IAP-I gene, were induced in the rat intestine by oral administration of LPS (29). The lipid A of LPS contains two phosphate groups and is known to be dephosphorylated by IAP (43, 44), resulting in its detoxification. IAP-I is therefore constitutive in the rat intestine and is a more primitive isoenzyme than IAP-II, and heat-inducible IAP-I as a dephosphorylating enzyme may play an important role in the host defense system against pathological stress (inflammation, infection, fever, or metals) or physiological stress (cell differentiation or tissue development).

Crystal structures of human AP isozymes, PLAP, and TNAP have been modeled and evolved a number of functional elements and properties not present in the *Escherichia coli* AP, on NH2-terminal α-helix, crown domain, and metal-binding domain (30, 31, 39).

---

**Fig. 6. Effect of glutathione (GSH) on activating protein-1 (AP-1) binding activity, cAMP response element-binding protein (CREB) binding activity, and the expression levels of heat-induced genes.**

IEC-18 cells were incubated at 43°C for 1 h after 1 h preincubation with 30 mM GSH. A: nuclear extracts (5 μg of proteins) were incubated in microwells previously coated with double-stranded oligonucleotides containing a consensus binding sequence for AP-1 or CREB. In competition assays (filled columns), the nuclear extract was mixed with 20 pmol of soluble double-stranded oligonucleotide (AP-1, 5′-CGCTTGATGAGTCAGCCGGAA-3′ and CREB, 5′-AGAGATTGCCTGACGTCAAGAGCTAG-3′) and the DNA binding assay was then performed. B: total RNA was prepared and analyzed by RT-PCR. Expression levels of the genes were calculated with the β-actin level as an internal control and are shown as percentages of the corresponding heat-shock group values. The data represent the means ± SD of 3 values.
TNAP, the loop of crown domain amino acids 405–435 is directly involved in the binding of collagen (5, 22, 39). From the structural analysis of active site and active valley, the target of PLAP is considered to be a phosphorylated protein (31). When the amino acid sequences of human AP isozymes are homologized to that of rat IAP-I, the high frequency of mismatches is recognized in the bottom of the active site valley region and the loop region but not the active site. However, it is necessary to build a reliable rat IAP-I model to discuss the specificity of substrate and the role of stress condition.

However, we do not yet know the physiological function(s) of IAP-I as a stress protein or the mechanism of IAP-I regulation. A study is currently underway to determine whether the coding sequence upstream of the sequenced IAP-I gene promoter contains some HSEs that act functionally.

We thank Dr. Yoshimasa Hamada for his encouragement, help, and advice.

REFERENCES


50. Xie Q and Alpers DH. The two isozymes of rat intestinal alkaline phosphatase are products of two distinct genes. Physiol Genomics 3: 1–8, 2000.