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Molecular Cloning and Sequence Analysis of Hepatic Lipase Gene in Yak*

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Abstract

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*In order to study the biological function of hepatic lipase (**hl**) gene in yak, the cDNA sequence encoding hepatic lipase from yak was cloned by RT-PCR method using gene specific PCR primers. The cloned cDNA fragment (1540 bp) contained a 1503 bp open reading frame, encoded 500 amino acids (AAs) with a molecular mass of 56.734 kDa. It showed a high level of sequence identity to **Bos Taurus** (99.40%), **Homo sapiens** (77.64%), **Oryctolagus cuniculus** (76.20%), **Mus musculus** (68.88%), **Xenopus laevis** (55.27%) and **Danio rerio** (47.69%). The **hl** gene was expressed only in liver tissue and not in heart, spleen, kidney and brain tissues. Further analysis of yak HL amino acid sequence implied that it contained a complete lipase active site (VHLIGYSLGA) ranging from 162 to 171 amino acid residues. It also contained two conserved domains, **conserved lipase** domain (18AAs-350AAs) in its N-terminal and the PLAT domain (353AAs-488AAs) in its C-terminal. The phylogenetic analysis showed that yak and **Bos taurus** were the closest species. The prediction of secondary structures indicated that HL of yak had the similar secondary structure with other isolated HL. The results of this study suggested that **hl** gene of yak was similar with **Bos taurus** and was expressed only in liver tissue.*

Key words: Yak, hepatic lipase, cloning, expression.

Introduction

Hepatic lipase (HL) is a key lipolytic enzyme that hydrolyzes phospholipids and triglycerides and affects the lipid composition of all lipoprotein classes yielding particles that are optimal for receptor-mediated uptake (Brunzell and Deeb, 2001). Hepatic lipase was

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synthesized and secreted by hepatocytes that bound extracellularly to parenchymal and endothelial cell surfaces in the space of Disse (Jansen *et al.*, 2002). In liver, HL activity plays an important role in plasma lipoprotein metabolism (Santamarina-Fojo *et al.*, 2004). In addition to liver, HL was also found in the adrenal glands, ovaries, plasma and several steroidogenic tissues (Verhoeven and Jansen, 1994) and plays many important roles in these tissues (Santamarina-Fojo and Haudenschild, 2000; Perret *et al.*, 2002). HL contains many isomers that are involved not only in lipoproteins metabolism but also related to many diseases, such as atherosclerosis and coronary artery disease and dyslipidemia (Rufibach *et al.*, 2006). In addition, HL played an important role in protection of life.

Yak (*Bos grunniens*) is a remarkable animal developed on the Qinghai-Tibetan Plateau that is often called the "roof of the world". Because of its adaptation to life on the plateau many adaptability changes may influence its genetic material (Gerald *et al.*, 2003). Although the HL gene has been cloned in *Bos taurus*, but there is no report related to the cloning of Yak *gapdh* cDNA fragment. Here we report the cloning of cDNA sequence of Yak *hl* gene, the bioinformatics analysis of this gene and the expression detection of *hl* gene in liver, heart, spleen, kidney and brain tissues.

Materials and Methods

Liver, heart, spleen, kidney and brain tissue samples of yak were collected from a newly slaughtered 3.5 years old healthy male yak in northwest plateau of Sichuan province. Each tissue sample was cut into 0.5-1.0 cm^3 cubes, rinsed by DEPC treated water. To avoid RNA degradation samples were frozen in liquid nitrogen immediately until further treatment. Total RNA was extracted from these tissue samples using RNA reagent kit (Takara Biotechnology Co., Ltd.) according to the instruction. The extracted RNA were treated with DNaseI to avoid contamination of total DNA that may disturb the results and all the RNA samples were dissolved in DEPC water, stored at -80C. The purity and concentrations of each RNA sample were detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

Total RNA (400 ng) were reverse transcribed by SMART technology (Takara Biotechnology Co., Ltd.) according to the instruction of the kit. The primer F: 5'-CCCAGGTGAAACAGAAATGGA-3' and primer R: 5'-ACACCAGGTCTTCATTAAAGCT-3' were designed and used to amplify the cDNA of yak's *hl* gene according to the *hl* gene mRNA sequence of cattle (*Bos Taurus*, accession number: NM001035410). The primary synthesized ds-cDNA from liver was used as template for PCR amplification. PCR was performed with the following program: 94C for 4 min followed by 35 cycles of 94C 1 min, 63C 1 min, 72C 90s, and a final extension for 7 min at 72C.

The amplified DNA fragments were isolated by 1.0% agarose gel electrophoresis and then were purified by using Agarose Gel DNA Purification Kit (Takara Biotechnology Co., Ltd.). The purified PCR product was cloned into the pMD18-T vector (Takara Biotechnology Co., Ltd.) and then transferred into the *E. coli* DH5α. The recombinant plasmid was named as pMD18-T-HL and then the inserted cDNA fragment was sequenced by Beijing Sunbiotech Co., Ltd.

The expression pattern of *hl* gene was detected in different tissues by RT-PCR method. Total RNA of heart, spleen, kidney and brain were extracted and then synthesized into ds-cDNA by the same method used in previous step. Each ds-cDNA sample was used as the template in RT-PCR analysis and the reaction system and PCR procedure were the same as *hl* amplification procedure in liver tissue. *β-actin* (*Bos Taurus*, accession number: BT030480) was used as loading control in this experiment. *β-actin* primer F: 52 - GATGTGGATCAGCAAGCA-32 ; primer R: 52

-CCTTCACCGTTCCAGTTT-32. PCR was performed with the following program: 94C for 2 min followed by 30 cycles (94C 30s, 52C 30s, 72C 30s), and a final extension for 7 min at 72C.

The sequenced cDNA fragment was compared to nonredundant (nr) database at the National Center for Biotechnology Information (NCBI; Bethesda, MD) using the Blastx algorithm. Aligment of the HL amino acid sequence with other six species, including *Bos Taurus*, *Homo sapiens*, *Oryctolagus cuniculus*, *Mus musculus*, *Xenopus laevis* and *Danio rerio* were downloaded from Genbank (Protein Id: NP_001030487, NP_000227, NP_001075501, NP_032306, NP_001107731 and AAI63963). Function-point analysis was conducted by online tools motifs-PROSITE (http://cn.expasy.org/tools/scanprosite/). The conserved domain was searched against the Pfam database (http://pfam.sanger.ac.uk/). The signal peptide of CPI was predicted using SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). The secondary structure of HL was predicted by online service (http://imtech.res.in/raghava/apssp/). The phylogenetic tree was drawn by DNAMAN software on the basis of amino acid sequence of HL in 7 species.

Result and Discussion

The expected length of the amplified *hl* gene fragment was 1540 bp. The results showed that a fragment around 1500 bp was obtained and inserted into pMD18T vector. The recombinant plasmid was digested by restriction endonuclease *Eco*RIII and *Hind*I, and detected by PCR amplification. The results indicated that both restriction enzyme digestion and PCR amplification can produce a 1500 bp length DNA fragment, which was *hl* gene of 1540 bp. An open reading frame spans over 1503 bp, encodes a protein of 500 amino acids. The deduced HL molecular mass was 56.734 kDa with an isoelectric point of 9.21. Then the DNA sequence of *hl* gene was deposited in GenBank (accession no. EU780009).

The load control *β-actin* PCR product was 232 bp (Fig. 1). This observation suggests that the *hl* gene mainly plays important roles in liver tissue. *Hl* gene has been always considered as a functional gene and it could have potential applied value in biotic medicinal territory. This study confirmed that *hl* gene was expressed only in liver, but had no expression in heart, spleen, brain and kidney tissues of yak. It reflected such a fact that *hl* gene may have no role in some tissues although one researcher gave a further proof that HL enzyme in adrenal glands was observed only in newborn animals, but not in adults (Schultz *et al.*, 2000).

The derived sequence was compared with the public nucleotide and protein database (GenBank) using the Blastn and Blastx algorithms. Blastn analysis showed that the nucleic acid sequences were similar between yak and *Bos Taurus* HL (Table 1). The deduced amino acid sequence analysis showed that the

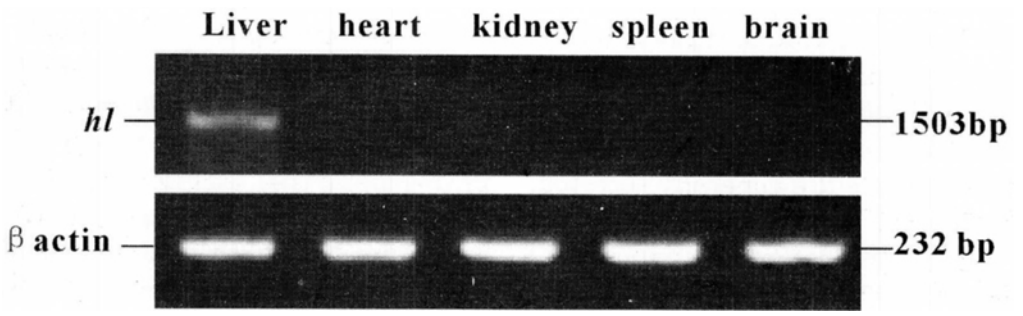

Fig. 1. Expression of *hl* gene in different tissues.

RT-PCR products amplified from liver, heart, kidney, spleen and brain, respectively by using *hl* and *β-actin* specific primers.

Table 1
Different nucleic acids and amino acids between *Bos grunniens* and *Bos Taurus*

Items	nucleic acids sites								amino acid sites		
	33	193	314	353	653	701	860	971	11	64	233
Bos grunniens	A	G	C	A	T	C	C	G	I	R	I
Bos Taurus	T	T	T	G	G	G	T	A	F	L	M

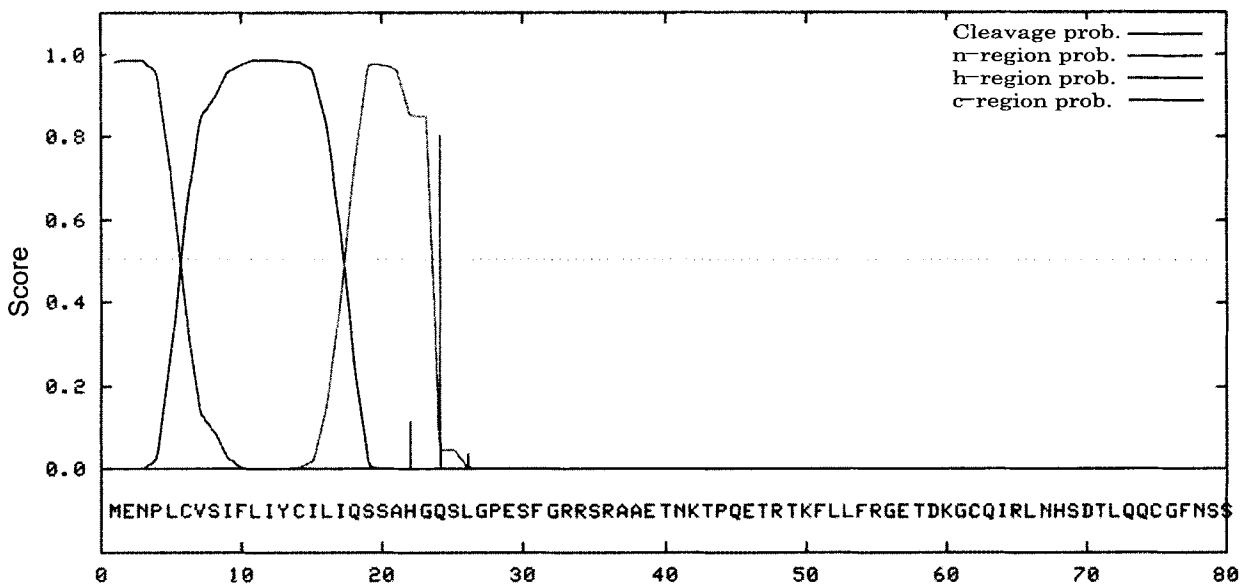

Fig. 2. Analysis of single peptide. Most likely cleavage site between position 23 and 24: AHG-QS.

identity of yak HL amino acid sequence with *Bos Taurus*, *Homo sapiens*, *Oryctolagus cuniculus*, *Mus musculus*, *Xenopus laevis* and *Danio rerio* were 99.40%, 77.64%, 76.20%, 68.88%, 55.27% and 47.69%, respectively.

The deduced amino acid sequence of HL suggested that mature hepatic lipase could be produced through cleavage at the conserved AHG-QS sites in the N-terminal regions in HL. Further analysis of yak HL amino acid sequence implied that it contains a signal peptide in its N-terminal (Fig. 2), the proteins with the signal peptide are generally secreted to the ecto-cell, may have important role as cytokines, which have potential application. Function-point analysis showed that the predicted 'VHLIGYSLGA' (162-171 sites) polypeptide was an active site of yak HL to be as a further proof that the cloned sequence was *hl* gene. Further analysis of yak HL amino acid sequence implied that it contains a conserved lipase domain (18AAs-350AAs) in its N-terminal and the PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain (353AAs-488AAs) in its C-terminal (Fig. 3).

In recent years, more and more hl genes have been cloned from animals, including *Bos Taurus*, *Homo sapiens*, *Oryctolagus cuniculus*, *Mus musculus*, *Xenopus laevis* and *Danio rerio*. The length of amino acids contained in these proteins ranged from 496 to 513 AAs. A rooted phylogenetic tree was calculated based on an alignment of amino acid sequences by using the maximum likelihood method implemented in the DNAMAN software. The results of phylogenetic tree (Fig. 4) analysis revealed that the phylogenetic relationship between HL of yak and *Bos taurus* was closest.

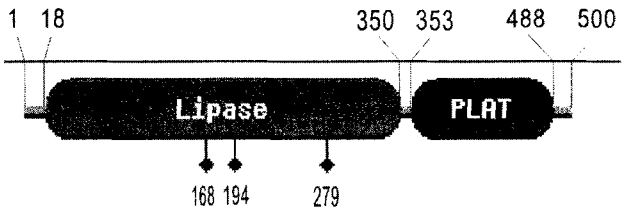

Fig. 3. The conserved domain of HL

N-terminal domain is a lipase domain that range from 18-350 amino acids and C-terminal domain is a PLAT domain which range from 353-488 amino acids. There are three active sites S168, D194 and H279.

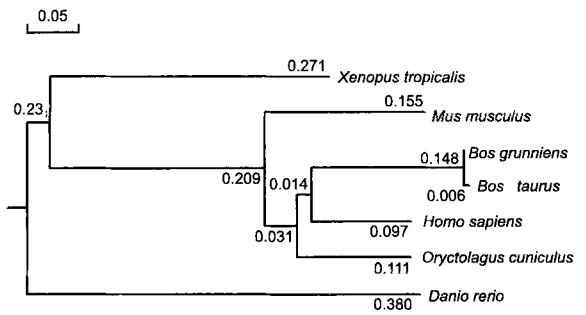

Fig. 4. Analysis of phylogeny (the number indicate branch length).

However, the HL biological function of yak has not yet been fully understood. Future work in our lab will aim at studying the biological function, protein expression and biomedical applications of *hl* gene in yak.

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एस. ली, एम. जीयान, वाई. लियु, एक्स. फु, जे. ड्रैकले, वाई. चेन, वाई. ये, वाई. वांग, जे. झांग। याक में याकृत लाइपेज जीन का क्लोनीकरण और अनुक्रम विश्लेषण।

याक में याकृत लाइपेज जीन की जैविक क्रिया अध्ययन हेतु याक के सीडीएनए अनुक्रम कोडन याकृत लाइपेज का जीन विशिष्ट पीसीआर प्राइमरों के उपयोग से आरटी-पीसीआर विधि से क्लोनीकरण किया गया। कोडीकृत सीडीएनए खंड (1540 बीपी) में एक 1503 बीपी मुक्त पठन फ्रेम कोडित 500 अमीनों अम्लों (एएएस) के साथ 56.734 केडीए की एक अणु राशि मिली। यह कुकुंदहीन गो पशु के साथ (90.40%) अधिक समानता दिखाता है जो कि मानव (77.64%), शशक (76.20%), मूषक (68.88%), जेनोपस लैइविस (55.27%) और डैनिओ रेशियों (47.69%) से अधिक थी। याकृत लाइपेज का केवल याकृत ऊतक में अभिव्यंजन था और हृदय, तिल्ली, वृक्क और मस्तिष्क ऊतकों में नहीं था। याक के याकृत लाइपेज के और विश्लेषण से अमीनो अम्ल अनुक्रम द्वारा ज्ञात हुआ कि इस में एक पूर्ण लाइपेज सक्रीय (वीएचएलआईजीवाईएसएलजीए) 162 से 171 अमीनो अम्ल अवशेष था। इसके दो संरक्षित प्रक्षेत्रों में एक एन सिरे पर लाइपेज प्रक्षेत्र (18 एए से 350 एए तक) और दूसरा इसके सी-सिरे पर पीएलएटी प्रक्षेत्र (353 एए से 488 एए तक) था। वंशावली विश्लेषण से याक सबसे निकट संबंध कुकुंदहीन गो पशु से है। द्वितीयक संरचना की प्रागुक्ति ने दर्शाया कि याकृत लाइपेज अन्य प्रजातियों के समान द्वितीयक संरचना वाला है। परिणामों से ज्ञात हुआ कि याक का याकृत लाइपेज बास टारस जैसा ही है और यह केवल यकृत ऊतक में अभिव्यक्त होता है।