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Evolutionary aspects of the gene for the classical enzyme polymorphism, ACP1

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Abstract

The gene for the classical polymorphic marker, ACP1, contains an unusual construction, two alternative exons, 3F and 3S, interspaced by a short 41-bp non-coding sequence, too short to function as a normal intron. During processing, two different mRNAs are produced, one containing 3F, the other 3S, resulting in the expression of two isoforms of the enzyme in human tissues. The fast and slow isoforms have different enzymatic properties and may have different physiological functions. This low molecular weight enzyme seems to function as a protein phosphotyrosine phosphatase (LMPTP). The LMPTP gene is highly conserved through evolution and has been found all the way back to yeasts, however, these express only one isoform. The ability to generate two functionally different isoforms by alternative splicing may be of evolutionary significance. We have analysed various species for LMPTP isoforms and gene structure. Two isoforms with different properties were observed in mammals and in fish, even in the evolutionary old shark. Exons similar to the human 3F and 3S were detected in cow, pig and cod, however, the intron between them is longer, 47 bp in cow and pig and 56 bp in cod.

Keywords: ACP1; LMPTP; Alternative exons; Isoforms

1. Introduction

The gene for the classical polymorphic marker, red cell acid phosphatase (ACP1), contains an unusual construction, two alternative exons, 3F and 3S, interspaced by a short 41-bp non-coding sequence, too short to function as a normal intron [1]. During processing, two different mRNAs are produced, one containing 3F, the other 3S. This

Abbreviations: LMPTP, low molecular phosphoprotein phosphatase.

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results in the expression of two isoforms of the enzyme in human tissues. The fast and slow isoforms have different enzymatic properties and may have different physiological functions. This low molecular weight enzyme seems to function as a protein phosphotyrosine phosphatase (LMPTP) [2]. The LMPTP gene is highly conserved through evolution and has been found all the way back to yeasts, however, these express only one isoform [3,4]. The ability to generate two functionally different isoforms by alternative splicing may be of evolutionary significance. To shed light on when in evolution this ability occurred we have analysed various species.

2. Material and methods

Animal and fish blood and tissues were obtained from The Royal Veterinary and Agricultural University, from Danmarks Akvarium and from freshly landed fish. Purification of LMPTP isozymes from the blood of cod was performed by affinity chromatography according to Ref. [5]. Size exclusion chromatography of isoforms was performed as described [6]. Amino acid sequencing of cod isozymes was performed using quadrupole time-of-flight mass spectrometry de novo sequencing. Isozyme electrophoresis was performed in starch gels as previously described [7]. Tests for activity modulation by purines were performed by inclusion of either 10 mM adenine or hypoxanthine in the staining solution which contained 4-methyl-umbelliferyl phosphate as substrate; the zymograms were viewed under UV-light. DNA was extracted by alkaline treatment [8]. cDNA was synthesised from RNA from cod tissue using standard protocols. Primers against bovine exon 2 and 3S were designed from the cDNA sequence (gi:163595) and were used to amplify bovine and porcine genomic LMPTP sequence. PCR was performed using standard protocols. Amino acid sequence data obtained on LMPTP from cod was used to design degenerate primers for amplification of cDNA from cod. PCR fragments were cloned using "Original TA-cloning Kit" (Invitrogen) and sequenced using universal primers. Sequenced cDNA fragments were used for the final design of primers for amplification and sequencing of genomic DNA.

3. Results and discussion

3.1. LMPTP isoforms

The presence of two LMPTP isoforms have previously been detected in various mammals and birds [9–11], but the gene structure has not been determined.

In the present study, we tested blood and tissues from mammals (cow and pig) and from fish (plaice, cod, eel, ray, shark), which are the common ancestors of birds and mammals. We also tested tissues from various invertebrates: mollusca (octopus, snail), annelida (earthworm) and cnidaria (sea anemone).

Two isoforms were detected in all the vertebrates, even in the evolutionary old shark (350 million years). Using size exclusion chromatography, it was confirmed that the isoforms were of similar low-molecular size as the human isoforms. It was further shown

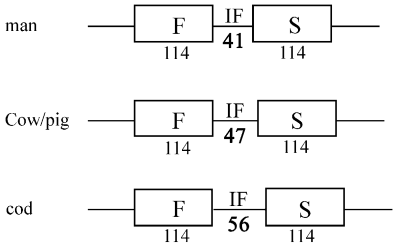

Fig. 1. Alternative "fast" (F) and "slow" (S) specific exons in the LMPTP gene in man, cow, pig and cod. The length (bp) of exons and introns is shown below.

that the two isoforms of each species responded differently to activity modulation by purines (adenine and hypoxanthine), which is characteristic of the human isoforms.

No distinct enzyme bands were observed with invertebrate tissues despite several electrophoretic buffer systems being tried. The invertebrates were not tested further.

3.2. Gene structure

3.2.1. Mammals

A segment of the LMPTP gene from cow and pig was amplified using primers against supposed exon 2 and exon 3S sequence as designed from published cDNA sequences. Amplicons of the expected lengths were obtained and sequencing showed a gene structure in these two mammals similar to the human. Exons 3F and 3S showed roughly 80% and 95% homology with the human sequences, respectively. However, the bovine and porcine intron IF is 47 bp compared to the human 41 bp (Fig. 1).

3.2.2. Fish

Several attempts to amplify cDNA from fish using low stringency PCR with primers based on the bovine amino acid sequence failed. It was therefore decided to purify LMPTP isozymes from fish and determine the amino acid sequence. This was accomplished using pooled blood from cod. The correct amino acid sequence allowed the construction of sets of nested degenerate primers. When using these with cDNA from cod, PCR products were obtained. Sequencing yielded information for the design of new sets of primers which allowed PCR of genomic DNA from cod. A segment from the putative exon 3F to exon 4 was obtained and was cloned and sequenced. The results show that the LMPTP gene in cod also has a structure with two alternative exons, each 114 bp in length, and a short intervening 56-bp segment (Fig. 1), it is noted that this segment is longer than that in mammals. The homology with the human sequence is 69% at both the DNA level and the protein level.

4. Conclusion

The unusual gene structure found in the human ACP1 gene, i.e. two alternative exons (114 bp) interspaced with a short anomalous intron, was also observed in other

mammals and in fish; the length of this intron increases from 41 bp in humans to 56 bp in cod (Fig. 1). The human gene generates two isoforms with different properties; such two isoforms were also observed in mammals and fish, even in the evolutionary old shark. The occurrence of alternative exons in fish pushes the origin of this gene construct back to or prior to early vertebrates.

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