Molecular analysis on the ODC antizyme from flounder (*Parlichthys olivaceus*)

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Abstract

Ornithine decarboxylase (ODC) is a key enzyme on the regulation of cellular polyamines. ODC antizyme is a protein that represses ODC through accelerating enzymatic degradation by the 26S proteasome. We have isolated two distinct antizyme cDNA clones (*AZS* and *AZL*) from a brain cDNA library constructed with flounder (*Paralichthys olivaceus*). *AZS* and *AZL* cDNA clones were encoding 221 and 218 residues long respectively and revealed 57.7% amino acids sequence identity. The presence of two antizymes mRNA were detected in brain, kidney, liver, and embryo.

Introduction

Ornithine decarboxylase (ODC) antizyme plays an important role in the control of intracellular levels of polyamines, such as putrescine, spermidine, and spermine, which are essential for cell proliferation and differentiation and the biosynthesis of which is catalysed by ODC. Binding of antizymes is the signal for the ODC degradation catalysed by the 26S proteasome[1]. Another function of antizyme is to inhibit the cellular uptake of polyamines. With the ability to suppress both polyamine biosynthesis and transport, antizyme effectively attenuates cellular polyamine levels.

The expression of antizyme genes requires a specific +1 translational frameshift. The amino-terminal portion is encoded by open reading frame 1 (ORF1), and the remainder is encoded by the overlapping ORF2 in the +1 reading frame.

Translation experiments *in vitro* showed that the frameshifting is stimulated by polyamines. This is the first example of the involvement of frameshifting in animal gene expression and is one of the only two known cases of regulatory frameshifting. In fish, Saito *et al*[2] reported that two zebrafish antizymes (*AZS* and *AZL*) have different

expression and activities. However, the knowledge of the molecular structure of antizyme in the marine fishes is extremely limited. Also, the nature of two antizymes in these fish and their roles in the control of polyamine pathway is still unclear. In the present study, we initially focus on the isolation of cDNAs encoding and characterize its expressions in adult tissues.

Materials and Methods

RNA Isolation and cDNA library construction

Total RNA was isolated with a TRIzol reagent (Invitrogen). The construction of the brain cDNA library was performed using a ZAP-cDNA® Synthesis Kit (Stratagene).

Screening of antizymes cDNA and DNA sequencing

Conserved nucleotide sequences of antizymes among the vertebrate species were determined using NCBI nucleotide and protein sequence database. Probe for screening antizymes was labeled with a DIG (digoxigenin) oligonucleotide 3' end labeling kit (Roche). DNA sequencing of the excised phagemid was performed by ABI PRISMTM DNA sequencing kit (Applied Biosystems) and determined with ABI 377 Genetic Analyzer according to the manufacturers instructions (Applied Biosystems).

Northern blotting

Total RNA (5ug) was separated by electrophoresis on a 1.5% formaldehyde gel. The RNA on the gel was transferred to a nylon membrane by capillary blotting. For detection of AZS and AZL antizyme mRNA the blotted membranes were hybridized with a DIG-labeled AZS and AZL probe that was synthesized by a polymerase chain reaction, respectively. Hybridization and detection performed using a DIG hybridization and detection Kit (Roche).

Results and discussion

We have cloned two antizymes using a flounder brain library. Two PCR products, about 1110 bp, 1500 bp, were obtained by PCR using AZ-F and LT primers. Both PCR products represented antizyme genes but there were much of sequence differences between two clones, suggesting two different antizyme genes. Consequently, using the probes made with both PCR products, we obtained several positive clones and analyzed their nucleotide sequences.

The flounder *AZS* gene has 1274 bp including an open reading frame are 5'- and 3'-untranslated regions, encoding 221 amino acid residues. The cDNA consists of 40 bp of 5'-untranslated region (UTR), 663 bp of coding region and 571 bp of 3'-UTR, followed by a poly (A) sequence. The flounder *AZL* gene has 1706 bp including an open reading frame are 5'- and 3'-untranslated regions, encoding 218 amino acid residues. The cDNA consists of 65 bp of 5'-untranslated region (UTR), 654 bp of coding region and 987 bp of 3'-UTR, followed by a poly (A) sequence.

The first AUGs would initiate translation of an ORF (ORF1) that overlaps the longer downstream ORF (ORF2) such that a +1 translational frameshifting event in the overlap would generate a protein product analogous to the products of antizyme genes from higher eukaryotes. The flounder antizymes have a high similarity in amino acid residues with other species, greater than 50% sequence identity. By this analysis flounder *AZS* shows 78.8% and 54.1% sequence identity with zebrafish *AZS* and *AZL*, respectively. Also, flounder *AZL* shows 56.6% and 73.1% sequence identity with zebrafish *AZS* and *AZL*, respectively.

The expression of antizyme mRNA species was examined by Northern blot analysis with probes specific for the two mRNA sequences. The results were expressed two antizymes all tissues investigated, but detected more *AZS* than *AZL* mRNA level.

Summary

We have isolated two distinct flounder (*Paralichthys olivaceus*) antizymes cDNA clones (*AZS* and *AZL*) from a brain cDNA library. Their sequences revealed that both clones required translational frameshifting for expression. Taking account of +1 frameshifting, *AZS* and *AZL* products were 221 and 218 residues long respectively and shared 57.7% amino acid identity. The expression of antizyme mRNA species was examined by Northern blot analysis with probes specific for the two mRNA sequences.

References

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