Universal PCR Primer for Calmodulin Gene Intron in Fish

Seinen Chow

Division of Pelagic Fish Resources, National Research Institute of Far Seas Fisheries, Orido, Shimizu 424-8633, Japan

(Received April 13, 1998)

Key words: universal primer, calmodulin gene intron, RFLP, fish population genetics

Exon primed intron-targeted PCR may have several advantages to investigate genetic polymorphism in nuclear genome. Adoption of conserved exon sequences for designing PCR primers appears to largely avoid priming site polymorphism which causes differential amplification of alleles within a locus. The same set of primers may function in very distant species. Furthermore, introns appear to accumulate a much greater degree of genetic polymorphism within and between species than exons. Calmodulin gene is known to encode a highly conserved calcium binding protein which regulates cell function. Actually, Corte-Real et al.1) designed universal PCR primers to amplify the 3rd intron of calmodulin gene, and applied the polymorphisms to survey the genetic population structure of marine musoids. This study introduces a pair of PCR primers which may amplify homologous intron regions of the calmodulin gene among distant fish species, and investigates genetic polymorphism in the amplified fragments.

The DNA sequence data of the calmodulin gene (CaMMII) of human and that (CaM-A) of Medaka Oryzias latipes were derived from Koller et al.2) and Matsuo et al.,3) respectively. Highly conserved nucleotide sequences observed in exons 4 and 5 between these distant species were adopted to design primers. The primer sequence designed in the 4th exon was 5'-CTGACCATGATGGCCAGAAA 3' (CALMex4F), and that in the 5th exon was 5'-GTTAGCTTCTCCCCCAGGTT-3' (CALMex5R). Using the standard phenol-chloroform method, crude nucleic acids were extracted from frozen or ethanol preserved muscles of chum salmon Onchorhyncus keta, lancetfish Alepisaurus ferox, swordfish Xiphias gladius, butterfly tuna Gasterochisma melampus, skipjack tuna Katsuwonus pelamis, yellowfin tuna Thunnus albacares, bigeye tuna T. obesus, northern bluefin tuna T. thynnus orientalis, walleye pollack Theragra chalcogramma, bigeye fish Naso brevirostris and pufferfish Fugu rubripes, and from human hair-root. The PCR reaction mixture contained 0.2 units of Taq DNA polymerase (Perkin-Elmer Cetus, Norfolk, USA), 0.2 mM of each dNTP, 1.0 µl of manufacturer's supplied 10 X buffer, 2 mM MgCl₂, 10 pmol of each primer and 10 to 50 ng of template DNA, in a final volume of 10 µl. Amplification was carried out with an initial denaturation at 95°C for one minute, followed by 30 cycles of amplification (denaturation at 95°C for 0.5 minute, annealing at 60°C for one minute and extension at 72°C for two minutes, with a final extension at 72°C for ten minutes). PCR products and those digested byendonuclease were electrophoresed on a 2.5% agarose gel (Biogel, BIO101 Inc., La Jolla, CA, USA) in TBE buffer (50 mM Tris, 1 mM EDTA, and 48.5 mM boric acid).

Result from PCR amplification is shown in Fig. 1. Eminent amplification of a single fragment was observed in salmon, butterfly tuna, skipjack tuna, swordfish, walleye pollack and human. Based on the human's data,2) the size of 4th intron plus priming exon regions was estimated to be 402 bp. An amplified fragment from human in this study was approximately 400 bp, strongly supporting that the primer pair used was exactly amplifying the target region at least in human. Furthermore, an amplified fragment from swordfish was cloned and sequenced, and the nucleotide sequence (data not shown) indicated the 5' and 3' regions of the fragments matched well with exons 4 and

Fig. 1. Results of PCR amplification targeting the 4th intron of calmodulin gene.

M: molecular marker (1 kb ladder, GIBCO BRL), lane 1: chum salmon Onchorhyncus keta, 2: lancetfish Alepisaurus ferox, 3: swordfish Xiphias gladius, 4: butterfly tuna Gasterochisma melampus, 5: skipjack tuna Katsuwonus pelamis, 6: yellowfin tuna Thunnus albacares, 7: walleye pollack Theragra chalcogramma, 8: unicorn fish Naso brevirostris, 9: human. Results from pufferfish Fugu rubripes having no amplified fragment, and bigeye Thunnus obesus and northern bluefin T. thynnus orientalis tunas having identical fragments with yellowfin tuna were not presented.

Address of corresponding author:
Fax: 0543-35-9642, Tel: 0543-36-6045, E-mail: chow@enyo.affrc.go.jp

Permission to reproduce this article or portion thereof is granted provided the following acknowledgment is included: Reproduced from Fisheries Science 64(6), 999-1000 (1998)
Fig. 2. RFLP obtained by Bst UI digestion for the amplified fragment in swordfish.

M: molecular marker (1 kb ladder, GIBCO BRL). Undigested PCR product was loaded on the 2nd lane.

5 of human and Medaka calmodulin gene, respectively. Faint amplification of another fragment was observed in lancetfish, three Thunnus tuna species, and unicorn fish, suggesting amplification from paralogous or unrelated loci in these species. No amplification was obtained in pufferfish even in the case of using lower annealing temperature (50°C).

Even when a single fragment was likely amplified, it would be necessary to ascertain whether a locus under consideration was strictly a single copy. Restriction site polymorphism analysis and subsequent investigation on fitness for the Hardy-Weinberg equilibrium may be the best assurance of amplification from a single gene locus. Since large number of swordfish individuals was available in the laboratory, investigation of restriction site polymorphism was performed in a swordfish sample (n = 34) collected in the Mediterranean Sea. Restriction fragment length polymorphism was observed only in Bst UI digestion out of fifteen 4-base cutter endonucleases used. Presence of two alleles within a single gene locus was presumed from the rather simple restriction profiles (Fig. 2). A fragment having no Bst UI site was designated A allele, and that having a site was designated B allele. Frequencies of A and B alleles of the Mediterranean sample were 0.279 and 0.721, and the observed and expected heterozygosities were 0.388 and 0.408, respectively. Chi-square analysis to test discrepancy between the observed and expected numbers of genotypes indicated that the sample was well in accordance with the Hardy-Weinberg equilibrium (χ²=0.087, P>0.75). These results support the use of this polymorphic intron within the calmodulin gene as Mendelian marker in the swordfish.

References