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T cell receptor beta chain from sea bream (*Sparus aurata*): Molecular cloning, expression and modelling of the complexes with MHC class I

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Abstract

The T cell receptor is a fundamental mediator of the adaptive immune responses, since TR αβ on T cells recognize foreign structures (peptides derived from processed antigens) bound to the major histocompatibility complex (MHC) on APC cells. In the present study, we report the cloning of six TRB chains cDNA sequences from gilthead sea bream (*Sparus aurata*), a fish of high economical impact in South Mediterranean aquaculture. The V-BETA domains have the canonical features of known teleost and mammalian TR V-BETA domains and have been divided in four different subgroups. A multiple alignment of the six sea bream TRB chains with other known TRB sequences was assembled and showed the conservation of the four cysteine residues involved in disulphide bonds and of some amino acids with an important role in the assembly and signalling of the TR αβ/CD3 complex. Real-time PCR analysis was used to investigate TRB basal expression, that was maximum in the thymus followed by gut, and TRB *in vitro* expression after stimulation with LPS or PHA-L at 4 and 24 h (only the 4 h stimulation with LPS gave a significant effect). Moreover, the 3D structures of sea bream TRB chains and MHC-I were predicted by homology modelling with the final aim to investigate the interaction surface in the V-BETA/MHC-I complexes.

Keywords: T cell antigen receptor; TRB; *Sparus aurata*; Real time PCR; 3D structure; MHC class I

1. Introduction

Cell-mediated immunity is one of the main lines of defence that vertebrates rely on for eliminating pathogen agents. The T cell receptor (TR), the antigen receptor expressed by the T lymphocytes, is present in all jawed vertebrates, while it appears to be absent in jawless fish (Klausner et al., 1990). The TR is an heterodimer, consisting of either α/β or γ/δ chains which are members of the immunoglobulin superfamily (IgSF). Each chain contains a variable (V) and a constant (C) domain (Lefranc and Lefranc, 2001). T cells are activated when a TR heterodimer (αβ or γδ) in conjunction with the CD3 complex specifically recognizes an external antigen (Ag). TR αβ recognizes a processed Ag as a peptide presented by the major histocompatibility complex (MHC) expressed on antigen presenting cells (APC), while TR γδ is not MHC-restricted and appears to recognize Ag in a similar manner to the direct processes of Ig (Chien et al., 1996).

The TR specificity is determined by the three hyper-variable regions of the V domains: the complementarity determining regions (CDR) (Rast and Litman, 1994). The diversity of TR αβ is generated by the assembly of V, D and, for β chain, J genes (Lefranc and Lefranc, 2001). Recombination signal sequences and recombination activator genes (RAG) are required for rearrangement of the TR genes (Marchalonis et al., 2002). The analysis of the TR chain sequences led to the correct prediction that they would share a domain organization and binding mode similar to those of antibody Fab fragments (Warr et al., 1979; Davis and Bjorkmann, 1988; Claverie et al., 1989). The first crystal structure of TR αβ bound to a peptide/MHC complex (TR/pMHC) was obtained in 1996 (Garboczi et al., 1996) and 24 TR/pMHC (both TR/pMHC-I and TR/pMHC-II) are available at the moment (Rudolph et al., 2006) and are annotated in the IMGT/3Dstructure-DB database (Kaas et al., 2004).

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TRA and TRB genes have been characterised in both teleost and cartilaginous fishes (Partula et al., 1995; Hordvik et al., 1996; De Guerra and Charlemagne, 1997; Wilson et al., 1998; Miracle et al., 2001; Wermenstam and Pilstrom, 2001; Hordvik et al., 2004; Imai et al., 2005) with the Japanese flounder (*Paralichthys olivaceus*) being the first species in which genes of the four TR loci have been identified (Nam et al., 2003). Moreover, it has been demonstrated that TR genes in teleost fish are organized in translocon type clusters (De Guerra and Charlemagne, 1997; Zhou et al., 2003) and, as in mammals, the TRA and TRD genes appear to be at the same locus on the genome (Wang et al., 2001; Fischer et al., 2002; Nam et al., 2003). In contrast to mammals, there is a high degree of polymorphism of the TRBC genes in the bicolor damselfish (Kamper and Churchill McKinney, 2002). Finally, the expression of TRB chain has been monitored during amoebic gill disease in rainbow trout to verify its possible up/down regulation (Bridle et al., 2006) and the spectra typing of TRB transcripts has revealed that the naive T cell repertoire is dramatically skewed during viral infection (Boudinot et al., 2001, 2002).

In the present study, we report the cDNA sequences of six TRB chains from sea bream (*Sparus aurata*), a fish of high economical impact in South Mediterranean aquaculture, and their expression pattern both at basal level and after *in vitro* stimulations. These results will add a new tool for studying the effects of vaccinations and immuno-stimulations on the sea bream immune system. Moreover, we analysed the cDNAs organisation and predicted, by homology modelling, the 3D structures of the TRB chains alone or complexed with the sea bream MHC-I.

2. Materials and methods

2.1. Sea bream TRB cloning and sequencing

Two degenerate primers (TCNEFR: 5′-SACRTGGTCRGGRTARAA-3′ and TCNERV: 5′-GCNGTKTAYTWCTGTGC-3′ where N = A, C, G, T; W = A, T; R = A, G; K = G, T; S = G, C; Y = C, T) corresponding to highly conserved regions of known TRB genes were used in RT-PCR on total RNA extracted with Tripure (Roche) solution from a juvenile sea bream (150 g of weight) thymus. The leukocyte cells were obtained following the procedures described in Scapigliati et al. (2001). RT-PCR was performed using Ready-To-Go RT-PCR Beads (Amersham Pharmacia). Reactions were conducted using the Mastercycler personal (Eppendorf). The cycling protocol was one cycle of 94 °C for 5 min, 35 cycles of 94 °C for 45 s, 48 °C for 45 s, 72 °C for 45 s, followed by one cycle of 72 °C for 10 min. PCR products (15 μl) were visualised on 1% (w/v) agarose gels containing ethidium bromide (10 ng/ml) using hyperladder IV (Bioline) as size marker. Controls for the presence of DNA contamination were performed using the RNA samples as template. DNA amplified by PCR was purified using the QIAquick Gel Extraction Kit (QIAgen), inserted into the pGEM-T Easy vector (Promega). Plasmid DNA from at least ten independent clones was purified using the Wizard Plus SV Minipreps DNA Purification System (Promega) and sequenced using MWG DNA Sequencing Services. Sequences generated were analysed for similarity with other known sequences using the FASTA (Pearson and Lipman, 1988) and BLAST (Altschul et al., 1990) programs.

Further primers were designed based on the initial sea bream TRB sequence for 5′- and 3′- rapid amplification of cDNA ends (RACE)-PCR (TCRFR1: 5′-CCACCAACAGTGAAAGTGC-3′ and TCRAFR2: 5′-CGTTGCTTTGTGTGATCAGC-3′; TCRRV1: 5′-GCTGATCACACAAAGCAACG-3′ and TCRRV2: 5′-GCACTTTCACTGTTGGTGG-3′). cDNA was synthesised from total thymus RNA with the First-Strand cDNA Synthesis kit (Amersham Pharmacia) following the manufacturers instructions. For 3′ RACE-PCR, cDNA was transcribed using an oligo-dT adaptor primer (5′-CTCGAGATCGATGCGGCCGCT$_{15}$-3′). PCR was performed initially with the TCRFR1 primer and the oligo-dT adaptor primer, followed by a semi-nested PCR using TCRFR2 primer and the adaptor primer (5′-CTCGAGATCGATGCGGCCGC-3′). For 5′ RACE-PCR, cDNA was transcribed from total RNA using the oligo-dT primer, treated with *E. coli* RNase H (Promega), purified using a PCR Purification Kit (QIAgen), and tailed with poly(C) at the 5′ end with terminal deoxynucleotidyl transferase (TdT, Promega). PCR was performed initially with TCRRV1 primer and an oligo-dG primer (5′-GGGGGGIGGGIIGGGIIG-3′), and then semi-nested with TCRRV2 and the oligo-dG primers. Sequencing and similarity searches were as described above.

The obtained sequences were analysed for the presence of a signal peptide, using SignalP software (Nielsen et al., 1997), and of N- (with the NetNGlyc 1.0 Server) and O-linked glycosylation sites (Julenius et al., 2005). The V and C domains of the TRB chains were also analysed following the IMGT® (the international ImMunoGeneTics information system®) standardisation numbering (Lefranc and Lefranc, 2001; Lefranc et al., 2003, 2005b). Comparison of the sea bream TRB amino acid sequences to their counterparts from other species was carried out using the MEGA 3.1 Software (Kumar et al., 2004). Phylogenetic trees was constructed by the "neighbour-joining" method using MEGA 3.1 Software (Kumar et al., 2004) and bootstrap values were calculated.

2.2. Basal TRB expression analysis

To study the TRB basal expression, ten sea bream juveniles were sampled and leucocytes from different tissues (thymus, spleen, liver, gills, head kidney, gut) obtained as described in Scapigliati et al. (2001). Total RNA was isolated from each tissue separately with Tripure (Roche) following the manufacturer's instructions, resuspended in DEPC treated water and used for real-time quantitative PCR without pooling the samples coming from the different fishes. Controls for the presence of DNA contamination were performed using β-actin primers that bracket an intron.

For reverse transcription, the BioScript RNase H minus (Bioline) enzyme was used with the protocol described in Buonocore et al. (2007). The expression level of TRB transcript was determined with a Mx3000P™ real time PCR system (Stratagene) equipped with version 2.02 software and using the Brilliant