DNA binding domain of retinoid receptor gene (RXR) from a field crab inhabiting the Indian peninsula

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Retinoid receptors are ubiquitous throughout the animal kingdom both in invertebrates and vertebrates, including the humans. In vertebrates, the retinoid X receptor (RXR) is suggested to be responsible for a variety of functions including growth and reproduction. However, reports are very scarce on the exact function of the RXR in crustaceans. In the present paper, we report the sequence information on the DNA binding domain (DBD) of the retinoid hormone receptor gene (RXR) of a field crab, Paratelphusa sp. from the Indian peninsula. BLAST analysis of the RXR-DBD shows considerable sequence homology with other brachyuran crabs, such as, Uca pugilator, Gecarcinus lateralis and Carcinus maenas. Taking cues from the GenBank database, we could also draw out the identity of this domain of the RXR with major phylogenic groups, including insects, molluscs and vertebrates. The sequence analysis of the RXR-DBD of the crab revealed remarkable sequence similarity to crustaceans and vertebrates, as compared with those of the insects.

Keywords: Brachyuran crabs, Decapoda, DNA binding domain (DBD), nuclear receptor (NR), Paratelphusa, retinoid receptor (RXR)

Introduction

Retinoid X receptor (RXR), a member of the nuclear receptor (NR) super family, is suggested to play key roles in the hormonal regulation of both vertebrates and invertebrates. In humans, for example, several forms of RXR are known to exist to control various metabolic functions, including morphogenesis and cellular differentiation1. Recent studies reveal that RXR (and its ligands) could involve in anti-inflammatory activity in humans2. Among invertebrates, especially the insects, the ultraspiracle gene (USP) (a homolog of the RXR) encoding the retinoid receptor is suggested to regulate a wide variety of functions, such as, growth, reproduction and development3.

In crustaceans, which are phylogenetically close to insects, retinoids—the putative ligands for the RXR receptor—are known to be endogenous4. However, its exact role in crustacean system is still enigmatic. Studies on vertebrates reveal that the RXR share the typical domain structure characteristic of NRs5. Accordingly, the receptor molecule has 4 or 5 functional domains, such as, the highly variable A/B domain, the C-domain or the DNA-binding domain (DBD) that binds to the hormone-response element (HRE) situated in the target gene promoter, the linker D domain, the ligand binding E domain and the terminal F domain6. The studies conducted on vertebrates reveal that DBD also plays a pivotal role in receptor dimerization, an essential step in hormone action. Despite the previous attempts made on a number of vertebrates and invertebrates (especially the insects), nothing was known with respect to the hormone receptors in crustaceans until 1996. For the first time, DBD of the gene encoding the retinoid receptor (RXR) was isolated, cloned and sequenced from a crustacean, the fiddler crab (Uca pugilator)7,8. Subsequently, more investigations were made on crustacean DBDs including those of other brachyuran crabs (Gecarcinus lateralis & Carcinus maenas), shrimps (Marsupenaeus japonicus) and the branchiopod (Daphnia magna)8-13.

Having been the most conserved domain of the NR and having the ability to induce target gene expression through interaction with specific NR elements (NREs) present within the target gene promoters, DBD is considered to be one of the most functionally important domains in the hormone receptor5,6. Further, its involvement in dimerization with the other partner receptors (for instance, the ecdysteroid receptor, EcR) has attracted the attention of molecular endocrinologists. To our knowledge, this

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domain has been sequenced only in three brachyuran (true) crabs, viz., *U. pugilator* (GenBank Acc. No. U31832)\(^7\), *G. lateralis* (DQ067286)\(^9\) and *C. maenas* (EU683889)\(^10\), inhabiting North American shores. Significantly, no reports are available with respect to the hormone receptors of the crustaceans inhabiting the Indian peninsula, despite that it houses a rich fauna of this class with highly diverse habits and habitats. In the present paper, we present the first ever report on the sequence information of the DBD of an Indian variety of field crab *Paratelphusa* sp. The paper also deals with the results of our comparative study using bioinformatics tools to find homology with other phylogenic groups.

**Materials and Methods**

**Specimen Collection**

Female adult crabs (*Paratelphusa* sp.), used for the present study, were collected through conventional bait technique from the rice farms and adjacent areas of the Vellore Institute of Technology University Campus, Vellore (12.56° N; 79.8°E), India. The specimens were maintained in the laboratory in plastic cisterns, laid with wet sand. In most of the instances, the crabs were sacrificed as soon as they were brought to the laboratory.

**Tissue Collection for RNA Isolation**

The crabs were dissected in crustacean physiological saline\(^14\). The ovary, being the putative target for RXR, was chosen (from reproductively active organism in early vitellogenic stage with yolky oocytes of 150-200 µ size) as the source. The pair of ovaries was dissected out by cutting open the dorsal side of the carapace. Ovarian tissue (100 mg) was homogenized in TRIzol (Invitrogen, Carlsbad, CA, USA) reagent and the total RNA was extracted and precipitated by TRIzol-chloroform-isopropanol method\(^15\). The purity of the RNA was assessed through UV spectrophotometry (at 260 nm) and agarose gel electrophoresis. The isolated RNA was stored at −80 °C until further use.

**cDNA Synthesis and PCR Amplification**

The RNA obtained from the ovarian tissue was reverse transcribed into cDNA using Verso Enzyme cDNA Synthesis kit (Abgene-Thermo Fisher Scientific, USA) as per manufacturer’s instructions. The primers specific for the RXR gene were designed using the Primer III software and validated through Primer-Blast. Previous reports on the RXR sequence of the fiddler crab, *Uca pugilator* were also used as the cue for primer design\(^7,8\). Accordingly, the forward primer (5′–AGC CTC CGG TAA GCA CTA TG-3′) and the reverse primer (5′–CAC TTT TCA TCC CCA TGG TC–3′) were used for PCR amplification of the domain. Each 25 µL reaction mixture contained 50 ng of template DNA, 1 µL reaction buffer (with 1.5 mM MgCl\(_2\)), 2.5 mM dNTP, 1 µM each forward (F) and reverse (R) primers and 1 U of Red Taq DNA polymerase (Merck-Bangalore GeNei™, Bangalore, India). PCR mixtures were incubated in Veriti™ Thermal Cycler (ABI, USA) with an initial denaturation (95°C, 10 min), final denaturation (95°C, 1 min) and 30 amplification cycles with 60°C (45 sec) as annealing temperature and 72°C (30 sec) as extension temperature for each cycle. The last cycle had a longer extension period (72°C, 10 min). 8 µL of the amplified product was agarose gel (1.5%) separated at 50 V in Tris-borate-EDTA buffer (pH 8.3). The band was visualized with ethidium bromide (Sigma-Aldrich, St. Louis, MO, USA) and monitored using the Vilber Leumart (France) Gel Documentation System. The PCR band eluted using the gel purification kit (Qiagen, Germany) was sequenced at the facility of Chromous Biotech Ltd, Bangalore and deposited to GenBank (Acc. No. JQ239171). The sequence information was further subjected to Nucleotide BLAST (Blastn) and CLUSTALW (v5.0) alignments for assessing the extent of homology with other phylogenic groups.

**Phylogenetic Analysis**

For phylogenetic analysis, the sequence of the PCR amplified band was aligned using CLUSTALW in the MEGA v5.04 software. Further, the sequences were subjected to DNA maximum likelihood program with the bootstrap phylogeny test of 1000 replicates so as to obtain the phylogenetic tree.

**Results and Discussion**

**Amplification and Sequencing of RXR-DBD of Paratelphusa sp.**

The PCR amplification of the cDNA and subsequent agarose gel (1.5%) separation of the product gave a distinct band between 200 to 300 bp (Fig. 1). Upon gel elution (Qiagen) and subsequent sequencing, the band obtained was shown to contain a sequence of 225 bp (Fig. 2a). However, a closer analysis using the coding sequences from other brachyurans (for instance, *U. pugilator*) has permitted us to have a better picture of the sequence, as represented in Fig. 2b.
 Phylogenetic Analysis of \textit{Paratelphusa} sp.

The phylogenetic analysis of RXR-DBD of \textit{Paratelphusa} sp. was performed with RXR coding sequences (mRNA) of other brachyurans (\textit{U. pugilator}, \textit{C. maenas} & \textit{G. lateralus})\textsuperscript{7,9,10}, non-brachyuran decapods (\textit{M. japonicus} & \textit{Cragon crangon})\textsuperscript{5,12}, insects (\textit{Drosophila melanogaster}, \textit{Bombyx mori}, \textit{Aedes aegyptii} & \textit{Manduca sexta})\textsuperscript{16-18}, mollusc (\textit{Biomphalaria glabrata}), chordates (\textit{Danio rerio})\textsuperscript{10} and the \textit{Homo sapiens} (Kaighin \textit{et al} unpublished, cited in GenBank acc. no. HQ692843) with MEGA v5.04 software and the results are shown in Figs 3 & 4. The analysis showed that \textit{Paratelphusa} sp. forms a close cluster with other brachyurans and non-brachyuran decapods, implicating a closer evolutionary relationship with them. BLAST and CLUSTAL W analyses revealed that the DBD of \textit{Paratelphusa} sp. has about 90% identity with other brachyuran crabs, such as, \textit{U. pugilator} and \textit{G. lateralis}, while showing 88% identity with \textit{C. maenas}. The RXR-DBD of \textit{Paratelphusa} sp. is shown to have about 85% homology with the shrimp \textit{M. japonicus} (AB295493), but has only 78% homology with those of the brown shrimp \textit{Cragon crangon} (FJ231415)\textsuperscript{5,12}. The extent of homology seems to decrease as we drew comparisons with several of the non-crustacean arthropods, especially the insects. The RXR-DBD of \textit{Paratelphusa} sp. has only 76% identity with the USP-DBD of...
Manduca sexta (U44837), 75% with that of Aedes aegypti (AF305213) and 74% with that of Bombyx mori (NM001044005). Interestingly, the alignment studies further revealed that the RXR-DBD of Paratelphusa sp. is closer to the vertebrate RXR as to the insects. With the RXR-DBD of H. sapiens and, for instance, with that of the Giant Panda (Ailuropoda melanoleuca) (Tiffany et al unpublished, GenBank acc. no. XM002920705), this field crab has respectively shown 81 and 77% identity. To conclude, it is a matter of concern that several aspects of control mechanisms involved in decapod growth and reproduction are still unclear, especially in the context that hormonal up- and down-regulations are accomplished by the intricate balancing between the stimulatory and inhibitory principles in this class of arthropods. And, it is being increasingly realized that the studies on hormone receptor genes and its expression would help us unravel several of the ambiguities regarding hormonal actions. Thus, the information furnished in this paper could serve as a prelude to our understanding on the intricate mechanisms involved in the control of major metabolic events (growth and reproduction, for instance) in decapods. Pertinently enough, RXR in crustaceans is being suggested to be a putative receptor for the gonadotropin hormone, such as, methyl farnesoate, a terpenoid compound and homolog of the insect JH III. In the present study, we could detect the RXR transcripts from the growing ovary, implicating that the ovary could be a target for the receptor gene. Future research involving season-dependent and stage-dependent gene expression of the hormone receptor genes could provide us with valuable clues on this subject.

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