FEBS Letters 584 (2010) 4458–4462

journal homepage: www.FEBSLetters.org

Ecdysone receptor homologs from mollusks, leeches and a polychaete worm

Michel Laguerre [a], Jan A. Veenstra [b,*]

[a] UMR 5248 CNRS, Institut Européen de Chimie et Biologie, 33600 Pessac, France
[b] Université de Bordeaux, CNRS CNIC UMR 5228, 33400 Talence, France

ARTICLE INFO

Article history:
Received 13 September 2010
Revised 4 October 2010
Accepted 6 October 2010
Available online 8 October 2010

Edited by Takashi Gojobori

Keywords:
Ecdysone receptor
Imposex
Lottia gigantea
Helobdella robusta
Capitella teleta

ABSTRACT

The genomes of the mollusk *Lottia gigantea*, the leech *Helobdella robusta* and the polychaete worm *Capitella teleta* each have a gene encoding an ecdysone receptor homolog. Publicly available genomic and EST sequences also contain evidence for ecdysone receptors in the seahare *Aplysia californica*, the bobtail squid *Euprymna scolopes* and the medicinal leech *Hirudo medicinalis*. Three-dimensional models of the ligand binding domains of these predicted ecdysone receptor homologs suggest that each of them could potentially bind an ecdysone-related steroid. Thus, ecdysone receptors are not limited to arthropods and nematodes.

1. Introduction

The insect molting hormone ecdysone was initially isolated and identified from 500 kg of silkworm pupae in 1954 [1]. It was subsequently shown to be a steroid hormone, and like vertebrate steroid hormones it acts through a nuclear receptor. However, unlike the vertebrate steroid hormone receptors, the ecdysone receptor belongs to the type II nuclear receptors forming a heterodimer with the RXR-homolog ultraspiracle [2].

The ecdysone receptor has generated much interest, not only because of its crucial roles in molting, development and reproduction in insects [2], but also because ecdysone agonists and antagonists that interfere with its proper functioning are useful as pesticides [3]. Ecdysteroids are not limited to insects but are also present in crustaceans and other arthropods and, unsurprisingly, ecdysone receptor homologs have been found in virtually all major arthropod groups. There are also reports on ecdysone in other invertebrates, e.g., mollusks and leeches, but these are often based only on immunoreactivity and co-elution experiments in high pressure liquid chromatography. Such data may well reflect the presence of ecdysteroids in these species, but they can also be environmental contaminants, as are testosterone and estradiol in insects. The presence of ecdysone in annelids, mollusks and helminths is, therefore, considered ambiguous [4,5].

If ecdysone were present in non-arthropod invertebrates, one would expect such species to have ecdysone receptor homologs able to recognize ecdysone or similar steroids. Phylogenetic analyses suggest that nematodes are closely related to insects. Although the particularly well known genome of *Caenorhabditis elegans* does not contain a gene encoding an ecdysone receptor, ecdysone receptor homologs were recently identified from a number of other nematodes [6–9]. Furthermore, the receptor of the nematode *Brugia malayi* was shown to form a functional heterodimer with both insect and nematode RXR homologs that could be activated by ecdysone and its analog ponasterone, proving convincingly that the ecdysone pathway does exist in nematodes [9].

We have recently analyzed the genome of the mollusk *Lottia gigantea* for neuropeptide encoding genes and found that the molluscan and insect peptidomes are more similar than generally realized [10]. This suggested that, perhaps, other endocrine pathways are also similar and led us to look for a gene that could encode a homolog of the insect ecdysone receptor in the same species. After finding such a gene we built a 3-dimensional model for the ligand binding domain of the predicted receptor using the experimentally determined structure of the *Heliothis virescens* homolog as a scaffold. The results suggest that this receptor is probably able to bind an ecdysone-like steroid. Evidence for similar receptors was also found in other mollusks, as well as leeches and a polychaete worm, suggesting that this steroid hormone signaling pathway may be widespread in protostomians.

* Corresponding author. Fax: +33 5400 008 743.
E-mail addresses: m.laguerre@iecb.u-bordeaux.fr (M. Laguerre), j.veenstra@cnic.u-bordeaux1.fr (J.A. Veenstra).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.10.004

2. Materials and methods

2.1. Database searches

The BLAST [11] package (blast-2.2.17-19) was downloaded from (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and used to analyze the assembled scaffolds of the genomes of the mollusk*L. gigantea*, the leech *Helobdella robusta* and the poychaete worm *Capitella teleta* downloaded from http://genome.jgi-psf.org/Lotgi1/Lotgi1. download.ftp.html, http://genome.jgi-psf.org/Helro1/Helro1. download.ftp.html and http://genome.jgi-psf.org/Capca1/Capca1. download. ftp.html. These genomes were searched for sequences showing similarity to the insect ecdysone receptor and gene models were constructed and compared with those already created by the Department of Energy Joint Genome Institute. Other predicted known ecdysone receptors were downloaded from GENBANK and EST sequences were searched for evidence of ecdysone receptor homologs in additional species.

2.2. Sequence comparisons and phylogenetic analysis

Alignment of sequences was performed with ClustalW software using standard parameters [12]. The multiple alignment used for PhyML [13] was created using MUSCLE [14]. Accession numbers of the sequences used are: *H. robusta*: [jgi|Helro1|108893]; *L. gigantea*: [jgi|Lotgi1|170342]; *C. teleta*: [jgi|Capca1|125155]; *Agelena silvatica*: ADB24 759; *Liocheles australasiae*: BAF85822; *Ixodes scapularis*: XP_0024 05625; *Amblyomma americanum*: AAB94567; *Ornithodoros moubata*: BAE45855; *Daphnia magna*: BAF49031; *Neomysis integer*: ACO92359; *Celuca pugilator*: AAC33432; *Crangon crangon*: ACO44666; *Marsupenaeus japonicus*: BAF75375; *Acyrthosiphon pisum*: ACR45971;*H. virescens*: CAA70212; *Spodoptera exigua*: ACA30302; *Manduca sexta*: ECR_MANSE; *Bombyx mori*: NP_001166846; *Choristoneura fumiferana*: AAC61596; *Plutella xylostella*: ABQ81864; *Plodia interpuctella*: AAR8 4611; *Chilo suppressalis*: BAC11713; *Omphysa fuscidentalis*: ABS0 0248; *Bradysia coprophila*: ACY80738; *Calliphora vicina*: AAG46050; *Ceratitis capitata*: CAA11907; *Drosophila melanogaster*: NP_724456; *Culex quinquefasciatus*: XP_001844581; *Chironomus tentans*: ECR_CHITE; *Aedes aegypti*: AAA87394; *Anopheles gambiae*: XP_320323; *Tribolium castaneum*: NP_001135390; *Leptinotarsa decemlineata*: BAD99296; *Tenebrio molitor*: CAA72296; *Anthonomus grandis*: ACK57879; *Blattella germanica*: CAJ01677; *Locusta migratoria*: AAD19828; *Pediculus humanus corporis*: XP_002430228; *Nilaparvata lugens*: ACO55652; *Pheidole megacephala*: BAE47509; *Camponotus japonicus*: BAF79666; *Apis mellifera*: NP_001152827; *Nasonia vitripennis*: NP_001152828; *B. malayi*: ABQ28 713; *Dirofilaria immitis*: ADC42111; *Haemonchus contortus*: ADD49 663; *Pristionchus pacificus*: ACY82385.

2.3. Model building

Calculations were performed on a SGI Octane workstation using Macromodel version 6.5 (Columbia University, New York) [15] or Insight II and Discover ver. 2000 (Accelrys Inc.). The homology building procedure was performed on a Linux PC using the software Modeller version 9v2 [16]. Thirty models were generated and the 5 lowest-energy conformers were chosen for further studies. Models were checked with MolProbity software [17] downloaded from http://molprobity.biochem.duke.edu/. The crude models were then submitted to a partial minimization using Discover software and CVFF force-field. The backbone was first fixed and the whole protein was submitted to 100 steps of Steepest-Descent followed by 1000 steps of Conjugate Gradient (CG). Then the backbone was unfixed and tethered with a force of 100 kcal/Å and 1000 steps of Conjugate Gradient were applied. At this stage the protein was superimposed to the X-ray structure of *H. virescens* ecdysone receptor (PDB code 2R40) with 20-hydroxy-ecdysone docked inside. The ecdysone receptor was subsequently removed leaving 20-hydroxy-ecdysone within the structure of the receptor of *L. gigantea*. 14-Deoxyecdysone, built within MacroModel from 20-hydroxy-ecdysone, was superimposed on 20-hydroxy-ecdysone which was removed afterwards, leaving finally 14-deoxyecdysone within the binding site of *Lottia*'s receptor. The whole complex was next minimized with tethering on protein backbone (1000 steps CG, 100 kcal/Å). The resulting complex was then submitted to two consecutive molecular dynamics runs of 50,000 steps each (first at 200 K and time-step = 1.5 fs and then 300 K and time-step = 1.5 fs).

3. Results and discussion

The genome of *L. gigantea* contains several nuclear receptors, one of which shows strong sequence similarity to the arthropod ecdysone receptors in both the DNA binding and the putative ligand binding domains (Fig. 1). Two independent ESTs (FC691674 and FC701413), both of which are derived from the male gonad, show that the putative *Lottia* ecdysone receptor gene is expressed. Such receptors are possibly generally present in mollusks as shown by the sequences of the ligand binding domains of its homologs as predicted by genomic sequences in *Aplysia californica* and an EST (DW258895) from *Euprymna scolopes* (Fig. 2a). The genomic sequence of *A. californica* shows gaps and there is likely an error in the contig (AASC02013084) containing the ligand binding domain; consequently, we were unable to reconstruct the entire sequence of its ecdysone receptor homolog by genome analysis.

Having found a putative ecdysone receptor in a mollusk with supporting evidence for a similar receptor in two other molluscan species, we then analyzed the genomes of the leech *H. robusta* and the polychaete worm *C. teleta* for similar proteins and found that both these genomes also encode a putative ecdysone receptor homolog (Fig. 1). Six EST sequences (EY328877, EY328878, EY336 359, EY336360, EY336843, EY336844), representing likely a single mRNA from the embryonic stage, demonstrate that the *Helobdella* gene is expressed, but no ESTs were found for the *Capitella* gene. Additional evidence for an ecydsone receptor in leeches is provided by an EST from *Hirudo medicinalis* (EY505259); the ligand binding domain predicted by this EST shows strong sequence homology with that of *Helobdella* (Fig. 2b). Phylogenetic analysis of these and other sequences yields a phylogram which confirms the relationships between the different species. It also puts the mollusks, leeches and the polychaete worm together with significant bootstrap support (Fig. 3). Their separate position within the phylogram and the presence of homologous genomic and/or EST sequences for some of them from related species excludes the, admittedly remote, possibility that these were somehow due to contamination of genomic material used for DNA sequencing.

The DNA-binding capability of these newly discovered nuclear receptors are not in doubt due to the extreme conservation of this part of these proteins, the interesting question is obviously whether the ligand binding domain will be able to bind ecdysone. Structural similarity between different steroid nuclear receptors does not necessarily mean that these proteins bind the same, or even structurally related hormones. Thus, from various molluscan species a nuclear receptor related to the vertebrate estrogen receptor has been identified, but this receptor appears to be constitutively active and does not bind estrogens [18,19]. Molecular modeling studies have shown that the *Octopus* estrogen receptor is unable to bind estrogens due to steric clashes [20]. In order to explore the question whether the receptors identified here might bind ecdysone or related steroids, we modeled the ligand binding domains on the model established for the ecdysone receptor.

At least 3 X-ray structures are available: 2R40 for *H. virescens* (Lepidoptera) [21], 2NXX for *T. castaneum* (Coleoptera) [22] and 1Z5X for *Bemisia tabaci* (Homoptera) [23]. The last structure