3D models of lamprey progesterone receptor complexed with progesterone, 7α-hydroxy-progesterone and 15α-hydroxy-progesterone

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A B S T R A C T
Sea lamprey, a basal vertebrate, contains a progesterone receptor [PR]. An unusual property of lamprey is that gonadotropin-releasing hormone induces synthesis of 15α-hydroxy-progesterone [15α-OH-P] instead of progesterone. There also is indirect evidence for 7α-OH-P in lamprey serum. To determine if there is a structural basis for the binding of 7α-OH-P and 15α-OH-P to lamprey PR, we constructed 3D models of the lamprey PR complexed with progesterone, 7α-OH-P and 15α-OH-P. These 3D models reveal that Met-277 in lamprey PR has a specific interaction with the 15α-hydroxyl on 15α-OH-P and with Met-192, which also contacts the 15α-hydroxyl group. We also find that 7α-OH-P has favorable contacts with side-chains in lamprey PR. BLAST searches reveal that Met-277 on lamprey PR is unique among vertebrate PRs. This unique site on lamprey PR could be a target for compounds to control reproduction in sea lamprey, an environmental pest in Lake Michigan.

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1. Introduction

Ancestors of lamprey evolved over 500 million years ago, placing lamprey at the base of the vertebrate line. This key evolutionary position of lamprey in vertebrates has motivated studies of lamprey to understand early events in vertebrate evolution [1–3], including the evolution of adrenal and sex steroid receptors [4–9]. Orthologs of the progesterone receptor [PR], estrogen receptor [ER] and a corticoid receptor [CR] have been cloned from the sea lamprey (Petromyzon marinus) [7] providing an opportunity to investigate the evolution of steroid binding to receptors in vertebrates [4,6,7,10–18].

An unusual property of sea lamprey is that 15α-hydroxy-progesterone (15α-OH-P), 15α-hydroxy-oestradiol (15α-OH-E2) and 15α-hydroxy-testosterone (15α-OH-T) appear to be the biological steroids, in contrast to P, E2 and T in humans [17,19–22]. Evidence for a biological role in lamprey for 15α-OH-P comes from its induction by gonadotropin-releasing hormone (GnRH) [23]. Interestingly, there is evidence for 7α-hydroxylase activity in sea lamprey [20], although neither 7α-OH-P nor other 7α-OH-steroids have been isolated from lamprey serum.

To determine if there is a structural basis for the binding of 7α-OH-P and 15α-OH-P to lamprey PR, we constructed 3D models of lamprey PR complexed with progesterone and 15α-OH-P and 7α-OH-P. These 3D models indicate that both 15α-OH-P and 7α-OH-P have favorable interactions with the side-chains in the steroid-binding domain of lamprey PR and human PR. Our 3D models identify a unique contact between Met-277 in lamprey PR and the hydroxyl group on 15α-OH-P. Met-277 also contacts Met-192, which has a van der Waals contact with the 15α-hydroxyl group. BLAST searches of GenBank reveal that Met-277 is unique to lamprey PR; in human PR, the corresponding residue is Leu-887, and other PRs in GenBank also contain a corresponding leucine. Thus, our 3D model of lamprey PR provides a structural explanation for the presence of 15α-OH-P in lamprey.

The uniqueness of the stabilizing interaction of lamprey Met-277 with 15α-OH-P suggests that it may be possible to find chemicals that selectively inhibit lamprey PR by using our 3D model of lamprey PR as a template for virtual screening of chemical libraries. Such chemicals could be used to control reproduction of P. marinus, which is a pest in the Great Lakes in the USA [24,25].

2. Experimental

2.1. Construction of 3D models

The 3D structure of human PR [PDB: 1A28] was used as a template for constructing the 3D model of lamprey PR [acces-
Interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

For acid, there are 79% positives with only one gap [Fig. 1]. This strong conservative replacements [e.g. arginine/lysine, aspartic acid/glutamic acid], there are 79% positives with only one gap [Fig. 1]. This strong similarity between lamprey PR and its template gives us confidence in the accuracy of our lamprey 3D model, which was constructed by Modeller [26]. We used the Multiple Mapping Method (MMM) software [27] to construct the 3D model of lamprey PR. We selected three alignment algorithms Muscle, Align2D and Clustal W to align lamprey PR with the human PR template [1A28]. MMM takes each alignment and constructs a composite alignment, which is then used by Modeller [26] to construct the 3D model of lamprey PR.

After we obtained the apo-3D model of lamprey PR, we inserted progesterone into lamprey PR, by overlapping lamprey PR with human PR. Progesterone was extracted from human PR and inserted into lamprey PR using the Biopolymer option in Insight II. Biopolymer also was used to add the 7α-hydroxyl and 15α-hydroxy groups to progesterone for analysis in lamprey PR and human PR.

We refined the structure of lamprey PR with P, 7α-OH-P and 15α-OH-P and human PR with 7α-OH-P and 15α-OH-P using Discover 3 in Insight II. For this energy minimization step, Discover 3 was run for 10,000 iterations, using the CVFF force field and a distant dependent dielectric constant of 2.

3. Results

As shown in Fig. 2, there is excellent overlap of the backbone of our 3D model of lamprey PR and the crystal structure of human PR.

The root mean square deviation [RMSD] of their Cx chains is 1.4 Å.

3.1. Comparison of progesterone binding to human PR and lamprey PR

In Fig. 3A and B, we show the interaction of progesterone with human PR and lamprey PR. As previously reported [28], in human PR, Gln-725, Arg-766 and Phe-778 have important stabilizing interactions with the A ring on progesterone. The C3-ketone is 3.2 Å from Ne2 on Gln-725 and 3.0 Å from Nε2 on Arg-766. The side chain on Arg-766 is stabilized further through a hydrogen bond between Ne and the backbone oxygen on Phe-778. Cβ2 on Phe-778 has a stabilizing van der Waals contact with C3 on progesterone. Interestingly, there is a stabilizing contact between Sβ on Met-801 and Cε3 on Phe-778. This stabilizing interaction between methionines and the π electrons on phenylalanine also is conserved in the 3D models of human PR with 15α-OH-P and 7α-OH-P, as well as lamprey PR complexed with P, 15α-OH-P and 7α-OH-P.

The D ring in progesterone is stabilized by interactions with Asn-719, Cys-891, Thr-894, Leu-797, Tyr-890, Phe-905 and Met-909. The C ring interacts with Met-756 and Met-759 and Met-801. Sβ on Met-801 has a van der Waals contact with Cε3 on Phe-778.

As shown in Fig. 3B, lamprey PR has similar stabilizing interactions with progesterone. For example, Gln-116, Arg-157 and Phe-169 stabilize the A ring. Sβ on Met-192 has a van der Waals contact with Cε3 on Phe-169. Similarly, most of the residues on lamprey PR that have stabilizing interactions with the C and D rings of progesterone align with residues on human PR that stabilize pro-

![Fig. 1. Alignment of lamprey PR with human PR. α-Helices and β-strands from the crystal structures of PR [PDB: 1A28] and notated below the alignment. Residues in human PR involved in binding of progesterone are shown in green. Residues in the steroid-binding domain in lamprey PR that differ from human PR are shown in pink. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)](image-url)
Fig. 3. Interaction of progesterone with human PR and the 3D model lamprey PR. (A) Interaction between progesterone and human PR. (B) Interaction between progesterone and the 3D model of lamprey PR. Lamprey PR has stabilizing interactions with the A ring of progesterone similar to those in human PR.

3.2. Comparison of 15α-OH-P binding to lamprey PR and human PR

Fig. 4A and B shows that human PR and lamprey PR have similar stabilizing hydrogen bonds with the A ring of 15α-OH-P, as

gesterone [Fig. 1]. This includes, Phe-280, which is a conservative replacement of Tyr-890 on human PR [Fig. 1]. An exception is Leu-188, which is distant from progesterone [Fig. 3A], in contrast to Leu-797 in human PR, which has a van der Waals contact with C15 [Fig. 3B].
Fig. 4. Interaction of 15α-OH-P with human PR and the 3D model lamprey PR. (A) In human PR, C2 on Leu-797 and Leu-887 and Cα on Met-801 have van der Waals contacts with 15α-OH-P. (B) Lamprey PR has stabilizing interactions with the A ring of 15α-OH-P that are similar to those in human PR. Met-192 has a van der Waals contact with 15α-OH-P. However, Leu-188 is 4.5 Å from 15α-OH-P, and thus does not have a stabilizing contact. Met-277 is 3.1 Å from the 15α-hydroxyl.

they have for progesterone. For example, in human PR, the C3-hydroxyl on 15α-OH-P is 3.3 Å from Nε2 on Gln-725 and 3.0 Å from Nη2 on Arg-766. Ne on Arg-766 is 3.1 Å from the backbone oxygen on Phe-778. Cα2 on Phe-778 has a van der Waals contact with C3 on 15α-OH-P. In lamprey PR, the C3-ketone is 3.4 Å from Ne2 on Gln-116 and 3.7 Å from Nη2 on Arg-157. Ne on Arg-157 is 3.9 Å from the backbone oxygen on Phe-169, which is 3.8 Å from C3.
Comparison of Fig. 4A and B, however, reveals an important difference between human PR and lamprey PR in their interaction with the 15α-hydroxyl on the D ring. In lamprey PR, S6 on Met-277 and C6 on Met-192 are 3.1 Å and 3.3 Å, respectively, from the 15α-hydroxyl. In contrast, in human PR, C62 on Leu-887, C6 on Met-801 and C62 on Leu-797 are 4.0 Å, 3.5 Å and 3.8 Å, respectively, from the 15α-hydroxyl group. Leu-188 in lamprey PR corresponds to Leu-797 in human PR. Unlike Leu-

![Diagram](image-url)

**Fig. 5.** Interaction of 7α-OH-P with human PR and the 3D model lamprey PR. (A) In human PR, C61 on Leu-887 and S6 on Met-801 have van der Waals contacts with 7α-OH-P. (B) Lamprey PR has stabilizing interactions with the A ring of 7α-OH-P that are similar to those in human PR. S6 on Met-192 and Met-277 stabilize 7α-OH-P. S6 on Met-277 contacts C6 on Met-192.
797, Leu-188 in lamprey PR does not contact the 15α-hydroxyl group.

3.3. Comparison of 7α-OH-P binding to human PR and lamprey PR

Fig. 5A and B shows that the stabilizing interactions between 7α-OH-P and human and lamprey PR are similar to that shown in Fig. 3A and B for binding of progesterone to human PR and lamprey PR, respectively. Thus, Gln-725, Arg-766 and Phe-778 in human PR and Gln-116, Arg-157, Phe-169 on lamprey PR have stabilizing interactions with the A ring on 7α-OH-P.

Interestingly, human PR and lamprey PR appear to have conserved methionine residues that interact with C7 and the C7-hydroxyl. Thus, in human PR, S0 on Met-801 is 3.1 Å from the 7α-OH group and S0 on Met-756 is 3.8 Å from C7, while in lamprey PR, S0 on Met-192 is 3.3 Å from the 7α-OH group and S0 on Met-147 is 3.7 Å from C7. A key difference is that in lamprey PR, S0 on Met-277 is 4.1 Å and 4.3 Å, respectively, from C7 and C7α-OH group [Fig. 5B]; the corresponding residue Leu-887 is 4.9 Å and 5.4 Å, respectively, from C7 and C7α-OH [Fig. 5A].

3.4. Met-277 lamprey PR is unique among vertebrate PRs

A BLAST search of GenBank, which contains over 100 PRs from a variety of vertebrates, found that all PRs contain a leucine that corresponds to Leu-887 in human PR [Fig. 1]. There were no vertebrate PRs with a methionine at this position. Moreover, adjacent to Met-277 are Gly-275 and Gly-276, which differ from Lys-885 and Val-886 on human PR. Gly-275 and Gly-276 provide a flexible tether, which should increase the conformations available for Met-277. Moreover, the methionine side chain can adopt many conformations or rotamers [29–31], in contrast to the side chain of Leu-887 on human PR. Fig. 6 shows this part of α-helix 10 in human PR and in the 3D model of lamprey PR complexed with 15α-OH-P. It is clear that this segment of lamprey PR and human PR has different structural conformations. In particular, methionines in lamprey PR have unique contacts with the 15α-hydroxyl or C7 on 15α-OH-P. Thus, in addition to a contact with the 15α-hydroxyl, Met-277 has contacts with Met-192, which also contacts the 15α-hydroxyl. Met-277 also has a van der Waals interaction with Met-147, which has a van der Waals contact with C7 [Fig. 6B]. In contrast, Leu-887 does not contact Met-801 [Fig. 6A], which corresponds to Met-192 in lamprey PR [Fig. 1].

Fig. 6. Comparison of a region in α-helix 10 of human PR and lamprey PR. The regions in α-helix 10 in human PR and lamprey PR that contain Leu-877 and Met-277, respectively, have different configurations due, in part, to the differences in the two amino acids that precede Leu-877 and Met-277, which provide more flexibility for Met-277 than for Leu-877. In addition, the side chain of Met-277 can adopt more conformations or rotamers than can Leu-877. (A) In human PR, Lys-885 and Gln-886, would be expected to limit the flexibility of Leu-877. (B) In lamprey PR, Gly-275 and Gly-276, which lack side chains, would be expected to provide flexibility for Met-277, which has contacts with 15α-hydroxyl group and side chains on Met-147 and Met-192.
4. Discussion

The key evolutionary position of lamprey as a basal vertebrate [1–3] stimulated us to investigate the structure of the steroid-binding site on lamprey PR. Lamprey serum is unusual in containing 15α-OH-P, which has been shown to be induced by GnRH [23]. There also is indirect evidence for 7α-OH-P in lamprey serum [20]. To determine if 7α-OH-P and 15α-OH-P have stabilizing interactions with lamprey PR, we constructed 3D models of lamprey PR complexes with 7α-OH-P, 15α-OH-P and progesterone using the crystal structure of human PR [28] as a template. The excellent conservation in the structure of human PR and our 3D model of lamprey PR, as seen in the RMSD of 1.4 Å between their Cα chains [Fig. 2] gives us confidence in our 3D models.

Analysis of the binding of 7α-OH-P, 15α-OH-P and progesterone to lamprey PR and human PR reveals that the interactions of the A ring of these three steroids with lamprey PR and human PR are conserved.

We find key differences between human PR and lamprey PR in their interaction with the C15-hydroxyl in the D ring on progesterone. In particular, there is a unique contact between Ser on Met-277 in lamprey PR and the 15α-hydroxyl on 15α-OH-P [Fig. 4B]. The corresponding amino acid in human PR is Leu-887, which has a van der Waals contact with the 15α-hydroxyl group [Fig. 4A]. Thus, comparison of the 3D model of lamprey PR with human PR identifies different chemical properties of the side chains on Met-277 and Leu-887 that contact 15α-OH-P.

Although 7α-OH-P has not been isolated from lamprey serum, Lowartz et al. [20] provided indirect evidence for 7α-OH-P in lamprey. Our 3D model of lamprey PR indicates that 7α-OH-P has favorable contacts with the side chains in the steroid-binding domain of lamprey PR [Fig. 5B]. Thus, if lamprey synthesizes 7α-OH-P, it would be expected to bind to lamprey PR.

Met-277 on lamprey PR is unique because other PRs in GenBank have a leucine at this position. Moreover, just upstream of Met-277 are two glycine residues in lamprey PR [Figs. 1 and 6]. Glycine is very different in structure and function from the Lys-885 and Gln-886, which are in the corresponding positions in human PR, supporting the importance of this region in steroid specificity of lamprey PR. Interestingly, there are three methionines in this region that have important interactions with either 15α-OHP or with each other. Met-277 has van der Waals contacts with Met-147 and Met-192, in addition to the contact with the 15α-hydroxyl on 15α-OH-P [Fig. 6B].

4.1. Evolutionary implications

Also of evolutionary relevance is the strong conservation of leucine at the corresponding position in human PR and in all other PRs in GenBank, which suggests an important role for Leu-877 in human PR. We also note that the position corresponding to Leu-877 in human PR also has a leucine in the GR [32], AR [33] and MR [34–36]. In addition to supporting the importance of this leucine in stabilizing the D ring of 3-keto-steroids, it suggests that this leucine was present in the common ancestor of 3-keto-steroid receptors.

Lamprey ER also has a unique methionine that is important in the binding of 15α-OH-estradiol [37]. The selective advantage for the evolution of 15α-hydroxylated steroids in sea lamprey is not known. Regarding the selective advantage of 15α-OH-P as the active progestin, one possible explanation is provided by the recent report [38] that 11-deoxycortisol is a corticosteroid in sea lamprey. This indicates that synthesis of active corticosteroids and progestins involves addition of either a C15α-hydroxyl or a C17α-hydroxyl, respectively, to P [Fig. 7], to form 15α-OH-P and 17α-OH-P, respectively. Hydroxylation at C21 of 17α-OH-P forms 11-deoxycortisol, the biologically active corticosteroid in sea lamprey [38]. Thus, addition of either 15α-OH-P or 17α-OH-P to P, partitions progestrone metabolism into pathways for ligands that...
activate either lamprey PR or CR. In this way, synthesis of 15α-OH-P instead of P, as the circulating progesterin in sea lamprey, would decouple steroid activation of lamprey PR from rapid increases in levels of 11-deoxy cortisol in response to stress.

An intriguing possibility, which is not incompatible with the above hypothesis, is that 15α-hydroxy-steroids evolved in parasitic lampreys, which during feeding on fish would be exposed to "conventional" sex steroids, such progesterone, testosterone and estradiol [J. Leatherland and S. Sower, personal communication]. The evolution of 15α-hydroxylated steroids and specificity for these steroids in their receptors would minimize the effects in parasitic lamprey of consumption of conventional steroids from fish. It will be interesting to determine if non-parasitic lampreys have 15α-hydroxy-steroids.

4.2. Environmental implications

Sea lamprey is a pest in the Great Lakes, where lamprey consumes trout and other valuable fish [24, 25]. Our 3D model of lamprey PR identifies a unique structure that interacts with the C and D rings on 15α-OH-P and 17α-OH-P. This difference from other vertebrate PRs could be exploited to find compounds that selectively inhibit lamprey PR by virtual screening of chemical libraries for binding to our 3D model of lamprey PR. Such contraceptives would provide a means to control sea lamprey.

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