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3D models of lamprey corticoid receptor complexed with 11-deoxycortisol and deoxycorticosterone

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ABSTRACT

The serum of Atlantic sea lamprey, a basal vertebrate, contains two corticosteroids, 11-deoxycortisol and deoxycorticosterone. Only 11-deoxycortisol has high affinity [$K_d \sim 3$ nM] for the corticoid receptor [CR] in lamprey gill cytosol. To investigate the binding of 11-deoxycortisol to the CR, we constructed 3D models of lamprey CR complexed with 11-deoxycortisol and deoxycorticosterone. These 3D models reveal that Leu-220 and Met-299 in lamprey CR have contacts with the 17α-hydroxyl on 11-deoxycortisol. Lamprey CR is the ancestor of the mineralocorticoid receptor [MR] and glucocorticoid receptor [GR]. Unlike human MR and human GR, the 3D model of lamprey CR finds a van der Waals contact between Cys-227 in helix 3 and Met-264 in helix 5. Mutant human MR and GR containing a van der Waals contact between helix 3 and helix 5 display enhanced responses to progesterone and glucocorticoids, respectively. We propose that this interaction was present in the CR and lost during the evolution of the MR and GR, leading to changes in their response to progesterone and corticosteroids, respectively.

1. Introduction

Ancestors of sea lamprey evolved over 500 million years ago, which places lamprey at the base of the vertebrate line. Lamprey's position as a basal vertebrate has motivated studies of lamprey to understand the evolution of vertebrates [1–3]. Sea lamprey (*Petromyzon marinus*) contains orthologs of the corticoid receptor [CR], progesterone receptor [PR] and estrogen receptor [ER] [4], which makes lamprey an important animal for understanding the evolution of vertebrate adrenal and sex steroid receptors [5–14].

Previously, we constructed 3D models of lamprey ER with various estrogens [15] and lamprey PR with various progestins [16]. These 3D models revealed that many structural interactions were conserved between estrogens in lamprey ER and human ER, and between progestins in lamprey PR and human PR. However, both lamprey ER and PR also contained some unique features that appeared to be important in the binding of 15α-hydroxy-estradiol to lamprey ER and 15α-hydroxy-progesterone to lamprey PR. The success of these previous studies motivated us to examine the binding of steroids to lamprey CR, which is the ancestor of the mineralocorticoid receptor [MR] and glucocorticoid receptor [GR] [4,7,17].

In addition to our interest in understanding how steroids bind to lamprey CR, we also were motivated by intriguing data from two reports on steroid binding to lamprey CR. In the first report, 100 nM of cortisol, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone (DOC) or aldosterone activated transcription in mammalian cells of the ligand-binding domain of lamprey CR fused to a GAL4-DNA-binding domain [7]. At concentrations below 10 nM, only aldosterone, corticosterone and DOC were transcriptionally active.

In the second report, Close et al. [18] found that 11-deoxycortisol and DOC were the two endogenous corticosteroids in lamprey serum. Neither cortisol nor corticosterone were present in lamprey serum. Further *in vitro* binding studies of native CR in lamprey gill cytosol revealed that only 11-deoxycortisol had high affinity [$K_d \sim 3$ nM] for lamprey CR. DOC, a mineralocorticoid, bound to CR with a substantially lower affinity than 11-deoxycortisol [18]. Moreover, neither cortisol, corticosterone nor progesterone bound to lamprey CR. These data and the evidence that the synthesis of 11-deoxycortisol in lamprey was stimulated by stress and that 11-deoxycortisol down-regulated synthesis of sex steroids and up-regulated gill Na^+K^+–ATPase prompted Close et al. [18] to propose that 11-deoxycortisol is the endogenous corticosteroid in sea lamprey.

With these reports [7,18] in mind, we constructed 3D models of lamprey CR complexed with 11-deoxycortisol and with DOC, the other corticosteroid in lamprey serum. We also constructed a 3D model of human MR with 11-deoxycortisol , which along with the crystal structure of the human MR with DOC [19], were compared with the 3D models of lamprey CR with 11-deoxycortisol and DOC. Analysis of our 3D models of lamprey CR and human

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MR with 11-deoxycortisol indicates that its 17α-hydroxyl has van der Waals contacts with Leu-220 and Met-299 on lamprey CR and with the corresponding Leu-766 and Met-845 in human MR. In contrast, DOC does not contact either Leu-220 in lamprey CR or Leu-766 in human MR.

During our analysis of the 3D model of lamprey CR, we uncovered an unexpected van der Waals contact between Cys-227 in helix 3 and Met-264 in helix 5 in lamprey CR. This van der Waals contact is absent between the corresponding residues in helix 3 and helix 5 in human MR [Ala-773, Ser-810] [20] and human GR [Gly-567, Met-604] [21]. The pathophysiological importance of this van der Waals contact was demonstrated by Geller et al. [20], who found that a Ser-810 to Leu mutation in human MR results in an interaction between Leu-810 and Ala-773, which changes progesterone from a mineralocorticoid antagonist to an agonist. Subsequently, Zhang et al. [21] showed that for the GR, mutation of Met-604 to leucine in helix 5 leads to a van der Waals contact with Gly-567 and an increased response to glucocorticoids. The presence of this helix 3–helix 5 interaction in the 3D model of lamprey CR and its absence in human MR and human GR leads us to propose that this interaction was present early in vertebrate evolution and lost later in the evolution of the MR and GR with important consequences for their response to 3keto-steroids.

2. Experimental

2.1. Construction of 3D models

The 3D structure of human MR co-crystalized with corticosterone [PDB: 2A3I] [22] was used as a template for constructing the 3D model of lamprey CR [Genbank: AAK20930]. The sequences of the steroid-binding domain of lamprey CR and human MR are 67% identical, and after including conservative replacements [e.g. arginine/lysine, aspartic acid/glutamic acid], there are 76% positives with no gaps (Fig. 1). This strong similarity between lamprey CR and its template gives us confidence in the accuracy of our lamprey 3D model [23]. We used the Homology option in Insight II to construct a 3D model of lamprey CR in the PDB format.

After we obtained the apo-3D model of lamprey CR, we inserted corticosterone into lamprey CR from human MR, by overlapping lamprey CR with human MR and extracting corticosterone from human MR for insertion into lamprey CR using the biopolymer option in Insight II. Corticosterone in lamprey CR and human MR was converted to 11-deoxycortisol by adding the 17α-hydroxyl and removing the 11β-hydroxyl. DOC was constructed from corticosterone by removal of the 11β-hydroxyl. We used the crystal structure of human MR with DOC [PDB: 2AA7] [19] to analyze the binding of DOC to the MR.

We refined the structure of lamprey CR with 11-deoxycortisol and DOC and human MR with 11-deoxycortisol 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 to approximate for water in the protein.

3. Results

In Supplemental Fig. 1, we show the overlap of the Cα-backbones of our 3D model of lamprey CR and the crystal structure of human MR. The root mean square deviation [RMSD] of their Cα chains is 1.2 Å, which indicates good overall similarity between our 3D model of lamprey CR and human MR.

3.1. Analysis of 11-deoxycortisol binding to the 3D model of lamprey CR

The interactions of key residues in our 3D model of lamprey CR with 11-deoxycortisol are shown in Fig. 2A. Gln-230, Arg-271, Phe-283, Cys-227 and Met-264 stabilize the A ring on 11-deoxycortisol. The C3-ketone on the A ring is 3.2 Å from Nε2 on

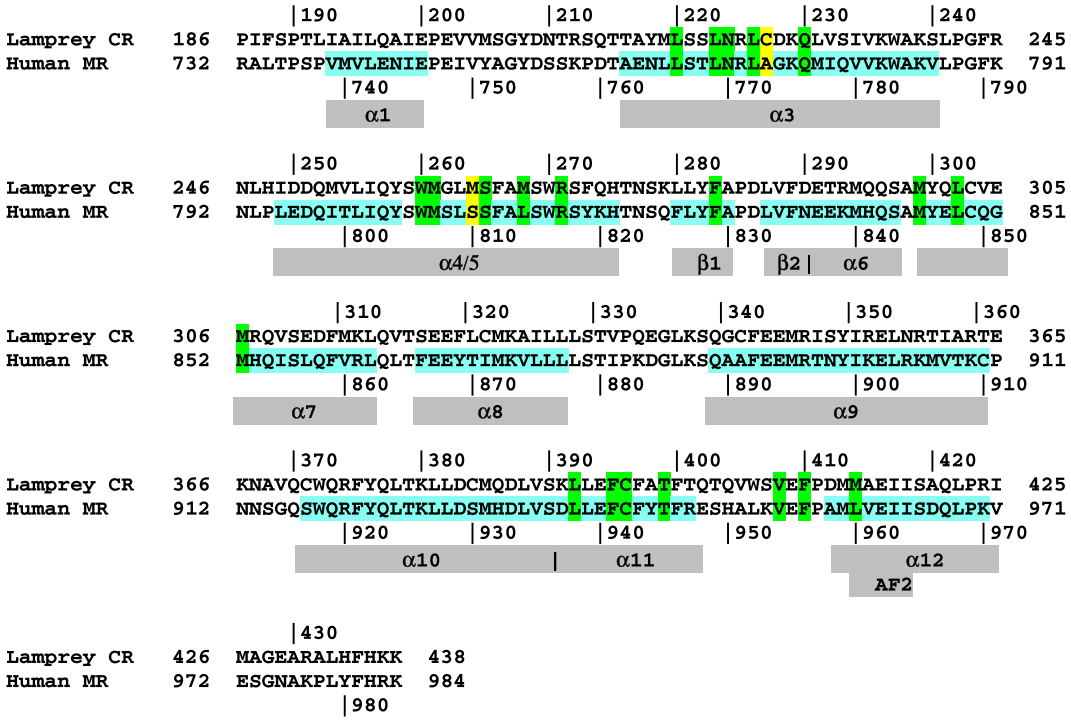

Fig. 1. Alignment of lamprey CR with human MR. α-helices and β-strands from the crystal structure of human MR [PDB: 2A3I] are shown in blue and notated below the alignment. Residues in human MR that contact corticosterone are shown in green [22]. The residues involved in the interaction between helix 3 and helix 5 in human MR in the 3dmodel of lamprey CR are shown in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)