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Solution structure of urotensin-II receptor extracellular loop III and characterization of its interaction with urotensin-II

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ARTICLE INFO

Article history:
Received 29 October 2007
Received in revised form
28 February 2008
Accepted 29 February 2008
Published on line 18 March 2008

Keywords:
UT receptor
Urotensin-II
Chemical shift perturbation
Interaction
NMR spectroscopy

ABSTRACT

Urotensin-II (U-II) is a vasoactive hormone that acts through a G-protein-coupled receptor named UT. Recently, we have shown, using the surface plasmon resonance technology that human U-II (hU-II) interacts with the hUT(281–300) fragment, a segment containing the extracellular loop III (EC-III) and short extensions of the transmembrane domains VI and VII (TM-VI and TM-VII). To further investigate the interaction of UT receptor with U-II, we have determined the solution structure of hUT(281–300) by high-resolution NMR and molecular modeling and we have examined, also using NMR, the binding with hU-II at residue level. In the presence of dodecylphosphocholine micelles, hUT(281–300) exhibited a type III β-turn (Q285–L288), followed by an α-helical structure (A289–L299), the latter including a stretch of transmembrane helix VII. Upon addition of hU-II, significant chemical shift perturbations were observed for residues located just on the N-terminal side of the β-turn (end of TM-VI/beginning of EC-III) and on one face of the α-helix (end of EC-III/beginning of TM-VII). These data, in conjunction with intermolecular NOEs, suggest that the initiation site of EC-III, as well as the upstream portion of helix VII, would be involved in agonist binding and allow to propose points of interaction in the ligand–receptor complex.

1. Introduction

Urotensin-II (U-II) is a disulfide-bridged peptide that was originally isolated from the goby *Gillichthys mirabilis* urophysis [5]. U-II isoforms were identified in many species of fish [6,14], as well as in amphibians [13] and mammals [15,16] including human [15]. Human U-II (hU-II) is characterized, as other isoforms, by a conserved cysteine-linked macrocycle CFWKYC but is the shortest isoform with 11 amino acids (ETPD [CFWKYC]V-OH) [15]. Several reports have revealed the potent vasoconstricting effects of U-II, and they described it as the most potent mammalian vasoconstrictor identified to date. However, the physiological role of U-II in the mammalian cardiovascular hemodynamics and vascular smooth muscle

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Abbreviations: CSD, chemical shift deviation; DPC, dodecylphosphocholine; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; EC, extracellular loop; GPCR, G-protein-coupled receptor; hUT, human urotensin-II receptor; URP, urotensin-II-related peptide; U-II, urotensin-II; gU-II, goby urotensin-II; hU-II, human urotensin-II; rUT, rat urotensin-II receptor; SAR, structure–activity relationship; TM, transmembrane domain.

0196-9781/$ – see front matter
doi:10.1016/j.peptides.2008.02.024

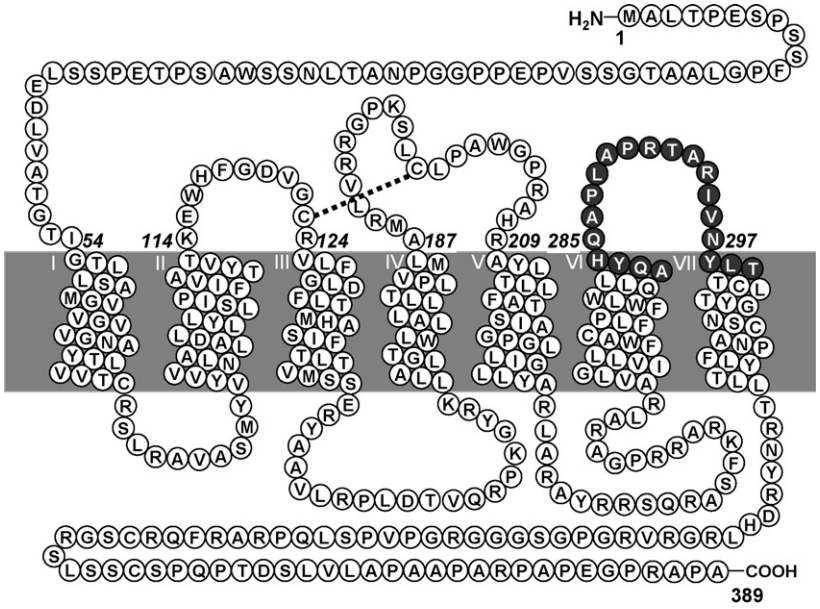

Fig. 1 – Two-dimensional representation of human UT receptor. Transmembrane helices are positioned according to Boivin et al. [7]. hUT(281–300) is shown in gray.

tone control is still not fully understood. For instance, diverse effects have been reported, from vasoconstricting to vasodilating responses [19]. Nevertheless, despite the heterogeneity of its vascular activities, U-II exerts a large array of physiological effects suggesting that it might be involved in pathological processes such as cardiovascular and renal diseases [55].

It has been shown that U-II action is mediated through a specific cell-surface receptor belonging to the superfamily of G-protein-coupled receptors (GPCRs). The U-II human receptor has been identified in 1999 [3,34] and is predominantly expressed in cardiovascular tissues and the motor neurons of spinal cord [3]. Previously known as an orphan G-protein-coupled receptor (GPR14), it is now designated as UT receptor [18]. This receptor belongs to class A, the rhodopsin-like family, and it contains 389 residues. It shares the GPCR common structure made of seven basic transmembrane domains, connected by extracellular and intracellular loops (Fig. 1).

Several data from structure–activity relationship (SAR) studies described the pharmacophoric requirements of U-II for receptor activation [17,23,29]. Hence, it has been shown that the N-terminal segment of the U-II molecule is not essential for the affinity and the activity, but that its conserved C-terminal cyclic core [CFWKYC] is necessary for high-affinity binding to UT receptor. Moreover, these studies demonstrated the importance of the Trp, Lys, and Tyr residues that are required for receptor recognition and activation [17,26]. NMR spectroscopy analyses of U-II in DMSO [22], water [17] and SDS [10] provided information about the precise spatial orientation of residues that are crucial for the interaction with the receptor. Thus, U-II structure shows that the N-terminal domain is in random coil, in contrast to the core region of the peptide that adopts a highly ordered compact conformation, induced by the disulfide bridge, with the presence on one side of the molecule of a hydrophobic cluster formed by the Phe, Trp and Tyr residues [22,26]. These data allow to postulate that this cluster may interact with a hydrophobic pocket located in the UT receptor or at its surface.

In contrast with U-II, because of its numerous SAR and conformational studies, our knowledge about the conformation and structural determinants of the UT receptor remains limited. Indirect methods such as bioinformatic techniques were used to explore UT conformation and ligand/UT complexes. The first theoretical model of gU-II/rUT was proposed by Kinney et al. [26]. They built a molecular complex in which the lysine residue of U-II was aligned towards the Asp130 side chain of transmembrane domain III. This structural domain arrangement allowed tight contacts between the receptor and the Trp, Lys and Tyr side chains of U-II. More recently, Lavecchia et al. have developed a model of peptidic or non-peptidic U-II agonist–hUT complexes, which gave similar information to those of Kinney et al. on the putative ligand-binding pocket [31]. Although both models offer a qualitative assessment of potential interactions of ligand/UT, they can only serve to generate hypotheses that must be validated by experimental data. Boucard et al. were the first to provide further data on the nature of the interaction between hU-II/rUT [8]. Using directed mutagenesis in combination with photolabeling techniques, they showed a close proximity of the core residues of U-II with the fourth transmembrane domain of rUT.

In the case of membrane proteins such as GPCRs, the traditional methods of NMR or X-ray crystallography are generally not applicable for obtaining high-resolution 3D structures. Alternative approaches are therefore required to get information on the receptor architecture and the structural characteristics that are involved in the recognition process. This is extremely important considering theoretical models, generated by homology modeling, provide little information