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Solution structure of amphibian tachykinin Uperolein bound to DPC micelles

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Abstract

Uperolein, a physalaemin-like endecapeptide, has been shown to be selective for Neurokinin 1 receptor. As a first step towards understanding the structure–activity relationship, we report the membrane-induced structure of Uperolein with the aid of circular dichroism and 2D ^{1}H NMR spectroscopy. Sequence-specific resonance assignments of protons have been made using correlation spectroscopy (TOCSY, DQF–COSY) and NOESY spectroscopy. The interproton distance constraints and dihedral angle constraints have been utilized to generate a family of structures using torsion angle molecular dynamics within program DYANA. The conformational range of the peptide revealed by NMR and CD studies has been analysed in terms of characteristic secondary features. Analysis of NMR data indicates that the global fold of Uperolein can be explained in terms of equilibrium between 3_{10}-helix and α-helix from residues 5 to 11. An extended highly flexible N-terminus displays some degree of order and a possible turn structure. A comparison between the structures of Uperolein and Substance P, a prototype and endogenous Neurokinin 1 receptor agonist, indicates several common features in the distribution of hydrophobic and hydrophilic residues. Both the peptides show an amphiphilic character towards the middle region. The similarities suggest that the molecules interact with the receptor in an analogous manner.

Keywords: NMR spectroscopy; Tachykinin; 3D structure; Circular dichroism; Bioactive peptide; Neurokinin receptors

1. Introduction

The tachykinin family is phylogenetically ancient and has been well conserved throughout evolution. Numerous structurally related peptides have been isolated from the mammals, birds, reptiles, amphibians, and fish, as well as from the invertebrates (Severini et al., 2002). The peptides of the tachykinin family so far isolated from the amphibian skin are grouped into two subfamilies, which have their prototypes in physalaemin and kassinin, respectively. Uperolein, a physalaemin-like endecapeptide, has been isolated from the methanol extracts of the skin of the Australian leptodactylid frogs *Uperoleia rugosa* and *Uperoleia marmorata* (Anastasi et al., 1975). Uperolein resembles physalaemin most closely both from the biological and a chemical point of view and is most dissimilar to Substance P (SP).[1] The other members of physalaemin subfamily include phyllomedusin and eledoisin. Amongst the non-mammalian tachykinins, physalaemin and Uperolein are the most active members of the family as reported on a number of test preparations, including rat salivary secretion. However, in the intensity of its hypotensive effect on the dog and rabbit blood pressure, Substance P surpasses all other tachykinins (Erspamer et al., 1975).

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[1] *Abbreviations used:* DPC, dodecylphosphocholine; CD, circular dichroism; TFE, trifluoroethanol; SDS, sodium dodecyl sulphate; DQF-COSY, double-quantum filtered correlation spectroscopy; 2D, two-dimensional; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotating frame Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; SP, Substance P; NKA, Neurokinin A; NKB, Neurokinin B; NPK, Neuropeptide K; NPγ, neuropeptide γ; NK1, Neurokinin 1; NK2, Neurokinin 2; NK3, Neurokinin 3; NMR, nuclear magnetic resonance.

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The mammalian tachykinins Substance P, Neurokinin A (NKA), and Neurokinin B (NKB) have similar activities as that of the non-mammalian members and have been more widely studied and characterized. The C-terminal region of tachykinins or message domain is believed to be responsible for activating the receptor whereas the divergent N-terminal region or the address domain varies in amino acid sequence and length and is postulated to play a role in determining the receptor subtype specificity (Schwyzer, 1987). The sequences of physalaemin subfamily have been reported in Fig. 1.

The tachykinins have been shown to elicit a wide array of activities such as powerful vasodilatation, hypertensive action, and stimulation of extravascular smooth muscle cells and are known to be involved in variety of clinical conditions including chronic pain, Parkinson's disease, Alzheimer's disease, depression, rheumatoid arthritis, irritable bowel syndrome, and asthma (Khawaja and Rogers, 1996). The broad spectrum of action of tachykinins is attributed to the lack of selectivity of tachykinins to their receptors. The three distinct G-protein-coupled receptor subtypes (designated as NK1, NK2, and NK3) have been cloned and characterized for tachykinins (Hanley and Jackson, 1987; Masu et al., 1987; Nakanishi, 1991). Uperolein has been shown to be selective for Neurokinin 1 (NK1) receptor subtype. While SP has a higher affinity for the NK1 type, NKA and NKB are so far reported endogenous ligands that exhibit the highest affinity for the Neurokinin 2 (NK2)- and Neurokinin 3 (NK3)-binding sites, respectively. The conformational features of tachykinins, which control receptor binding and influence their biological activity, are of significant interest, particularly as the selectivity of these peptides for different receptor sites is not fully understood.

Bioactive conformation of the tachykinin neuropeptides has been extensively investigated using high-resolution nuclear magnetic resonance (NMR), circular dichroism (CD), and infrared (IR) spectroscopy. The solution structure of SP, NKA, NKB, physalaemin, eledoisin, and various naturally derived or synthetic analogues has been reported in various membrane mimetic solvents (Convert et al., 1988, 1991; Ananthanarayanan and Orlicky, 1992; Seelig, 1992; Horne et al., 1993; Cowsik et al., 1997; Whitehead et al., 1998; Grace et al., 2001, 2003; Chandrashekar and Cowsik, 2003; Chandrashekar et al., 2004; Mantha et al., 2004; Dike and Cowsik, 2005, 2006). However, none of the reported data to date deals with the conformational analysis of Uperolein.

Substance P	**RPKPQQFFGLM-NH2**
Uperolein	**BPDPNAFYGLM-NH2**
Physalaemin	**BADPNKFYGLM-NH2**
Eledoisin	**BPSKDAFIGLM-NH2**
Phyllomedusin	**BNPNRFIGLM-NH2**

Fig. 1. Sequence alignment of physalaemin-like tachykinin peptides. "B" represents pyroGlu.

Binding of Uperolein to its receptor occurs in the membrane environment. Membrane contact is proposed to induce a specific conformation onto the peptide backbone before interacting with its receptor and this conformational alteration may be an essential step for the recognition by the receptor (Maurer and Ruterjans, 1994; Inooka et al., 2001). Although micelles are not perfect mimetics of lipid bilayers, a substantial number of structural studies on peptides and proteins bound to micelles have indicated that valuable structural information can be obtained from NMR studies of such systems (Lerch et al., 2004). Micellar systems have been used extensively in high-resolution NMR studies of peptide–membrane interaction as membrane mimics (Braun et al., 1983; McDonnell and Opella, 1993). Dodecylphosphocholine (DPC) micelles are one of the most widely used membrane mimics for such studies (Opella, 1997). High-resolution NMR spectra can be obtained on peptides bound to micelles of perdeuterated lipids, taking advantage of the effective isotropic reorientation of the micelle-bound peptides.

In the present study, we report the high-resolution three-dimensional structure of Uperolein bound to dodecylphosphocholine (DPC) micelles (PDB ID 2GFR), one of the well-characterized model membrane systems, using two-dimensional NMR spectroscopy. CD spectroscopy has been used to explore the secondary structural features of Uperolein in the presence of calcium ions and in different membrane mimetic environments, including 2,2,2-trifluoroethanol (TFE), TFE/water mixtures, sodium dodecyl sulphate (SDS) and DPC micelles. An attempt has been made to correlate the observed conformational differences to the binding ability and biological activity of various NK1 receptor agonists.

2. Materials and methods

2.1. Materials

Uperolein (pyroGlu-Pro-Asp-Pro-Asn-Ala-Phe-Tyr-Gly-Leu-Met-[NH_2]) was custom synthesized by Princeton Biomolecules (Langhorne, PA). The purity of the peptide reported by HPLC analysis was >98%. Perdeuterated DPC (d_{38}) was obtained from Cambridge Isotope Laboratories (Andover, MA). NMR reagents were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). TFE and SDS were obtained from Sigma (St. Louis, MO) in the highest available purity.

2.2. CD spectropolarimetry

Far-UV wavelength CD spectra of the peptide were recorded from 250 to 190 nm on Jasco J-720 spectropolarimeter (Jasco, Tokyo, Japan). The instrument had been