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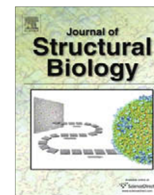

# Three-dimensional structure of Phyllomedusin, a NK1 receptor agonist bound to dodecylphosphocholine micelles

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ABSTRACT

Phyllomedusin, an amphibian tachykinin decapeptide, has been shown to be selective for Neurokinin 1 receptor. Because the micelle-associated structure may be relevant to the Phyllomedusin–receptor interaction, the three-dimensional structure of the Phyllomedusin in aqueous and micellar environments has been studied by two-dimensional proton nuclear magnetic resonance (2D $^1$H NMR spectroscopy) and distance geometry calculations. Sequence specific resonance assignments of protons have been made from 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 DYANA. The CD and NMR results show that, while in water Phyllomedusin prefers to be in an extended chain conformation, whereas in the presence of dodecylphosphocholine micelles, a membrane model system, a partial helical conformation is induced. Analysis of NMR data indicates that the global fold of Phyllomedusin can be explained in terms of equilibrium between $3_{10}$-helix and α-helix from residue 4 to 10. An extended highly flexible N-terminus displays some degree of order and a possible turn structure. A comparison between the conformational features of Phyllomedusin and different Neurokinin 1 receptor agonist indicates several common features in the distribution of hydrophobic and hydrophilic residues. The conformational similarities suggest that the molecules interact with receptor in an analogous manner.

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## 1. Introduction

Tachykinins are a family of closely related peptides that are actively involved in the central and peripheral nervous systems as well as in cardiovascular and immune systems of both lower and advanced life forms. The tachykinin family is phylogenetically ancient and has been well conserved throughout evolution. Mammalian tachykinins, Substance P (SP), [1]Neurokinin A (NKA) and Neurokinin B (NKB) are the earliest known members followed by the nonmammalian Physaelamine, Eledoisin and others. These peptides are characterized by a C-terminal consensus sequence, Phe-X-Gly-Leu-Met-$NH_2$. This C-terminal "message domain" is considered to be responsible for activating the receptor. The divergent N-terminal "address domain" varies in amino acid sequence and length and is postulated to play a role in determining the receptor subtype specificity (Schwyzer, 1987). Three distinct G-Protein coupled receptor subtypes have been cloned and characterized for tachykinins (Nakanishi, 1991; Masu et al., 1987; Hanley and Jackson, 1987) designated as NK1, NK2 and NK3 (Neurokinin, NK). All tachykinins interact with all the three receptor subtype with SP preferring NK1, NKA preferring NK2 and NKB preferring NK3.

Tachykinins have been shown to elicit wide array of activities such as powerful vasodilatation, hypertensive action and stimulation of extra vascular smooth muscle in mammals 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 wide range of physiological activity of tachykinins has been attributed to the lack of selectivity for a particular receptor subtype (Nakanishi, 1991) which may be related to the conformational flexibility of the short, linear peptide and thereby account for the lack of selectivity. 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.

Phyllomedusin is a linear decapeptide with amino acid sequence, PGlu-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-$NH_2$ and has a high affinity for NK1 receptor subtype. Phyllomedusin has been isolated from the dried skin secretion "sapo" of *Phyllomedusa bicolor*, a rare arboreal frog (Erspamer et al., 1986). Phyllomedusin

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[1] *Abbreviations used:* DPC, dodecylphosphocholine; CD, circular dichroism; TFE, trifluoroethanol; SDS, sodium dodecyl sulfate; 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; NK, Neurokinin; SP, Substance P; NKA, Neurokinin A; NKB, Neurokinin B; NK1, Neurokinin-1; NK2, Neurokinin-2; NK3, Neurokinin-3; NMR, nuclear magnetic resonance.

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has been shown to strongly affect the salivary glands, tear ducts, intestines and bowels, contributing to smooth muscle contraction (Anastasi and Erspamer, 1970). Also Phyllomedusin has a pronounced effect on the dilation of blood vessels that it may increase the permeability of blood–brain barrier thus facilitating access to the brain not only of itself, but also of other active peptides (Anastasi and Erspamer, 1970; Mignogna et al., 1997). Finding a key to unlocking the secret of passing that barrier is vital to the development of drugs for various neurological disorders such as Alzheimer's. Apart from being a potential drug candidate, elucidation of bioactive conformation of Phyllomedusin is expected to be helpful in understanding the structure–activity relationship of Neurokinin receptor and as a starting point for ligand based drug design.

Bioactive conformation of tachykinin neuropeptides has been extensively investigated using high-resolution nuclear magnetic resonance (NMR), circular dichroism (CD) and Infrared (IR) spectroscopy. The sequences of Physalaemin subfamily has been reported in Fig. 1. Solution structure for SP, NKA, NKB, Physalaemin, eledoisin and various naturally derived or synthetic analogs has been reported in various membrane mimetic solvents (Convert et al., 1988; Convert et al., 1991; Seelig, 1992; Ananthanarayanan and Orlicky, 1992; Horne et al., 1993; Cowsik et al., 1997; Whitehead et al., 1998; Grace et al., 2001; Grace et al., 2003; Chandrashekar and Cowsik, 2003; Mantha et al., 2004; Chandrashekar et al., 2004; Dike and Cowsik, 2005; Dike and Cowsik, 2006a; Dike and Cowsik, 2006b). But none of the reported data to date deals with the conformational analysis of Phyllomedusin. Further, the conformation of Phyllomedusin bound to DPC micelles determined in this study supports structure–activity and receptor–ligand interaction data available on NK1 agonists (Whitehead et al., 1998; Wilson et al., 1994; Cascieri et al., 1992).

Binding of Phyllomedusin to its receptor occurs in the membrane environment. The membrane is proposed to induce a specific conformation to the peptide backbone of Phyllomedusin before interacting with its receptor, and this conformational alteration should be an essential step for the recognition by the receptor (Brown, 1979; Lauterwein et al., 1979; Braun et al., 1983; Maurer and Rüterjans, 1994; Pellegrini et al., 1996; Cowsik et al., 1997; Grace et al., 2001; Grace et al., 2003). Micellar systems have been used extensively in high-resolution NMR studies of peptide/membrane interaction as membrane mimics. Since the pioneering work on melletin and glucagons in micelles (McDonnell and Opella, 1993; Opella, 1997), there have been a large number of NMR studies of peptide structures and their interaction in micellar systems (Braun et al., 1983; McDonnell and Opella, 1993; Opella, 1997; Kallick et al., 1995; Maurer et al., 1991). DPC micelles often are chosen as "membrane mimics" for studying peptide–membrane interactions by using high-resolution NMR techniques. The zwitterionic phosphatidylcholine head group of DPC is similar to that found in biological membranes and forms small micelles of 50–60 molecules that rapidly reorient in solution. 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 study reported here, circular dichroism and NMR spectroscopic techniques have been used to investigate the secondary structure of Phyllomedusin in different solvents. Effect of calcium ions on the conformation of Phyllomedusin has been investigated using CD spectropolarimetry. The three-dimensional structure of Phyllomedusin bound to DPC micelles has been reported for the first time and the structure calculated has been deposited in the Protein Data Bank (PDB ID 2NOR). Several homonuclear two-dimensional NMR techniques (Wüthrich, 1986), such as double quantum filtered spectroscopy (DQF-COSY), Total correlation spectroscopy (TOCSY) and nuclear overhauser effect spectroscopy (NOESY) have been utilized in deriving the complete proton resonance assignments for Phyllomedusin in the water and in lipid medium. The NOESY crosspeak volumes have further been used to determine the interproton distances in three-dimensional space. An ensemble of model conformations has been generated for Phyllomedusin in the lipid medium using the program DYANA (Güntert et al., 1997). Also the conformational features of Phyllomedusin in aqueous and perdeuterated DPC micelles have been described and compared. Further an attempt has been made to correlate the observed conformational features to the binding ability and biological activity of various NK1 receptor agonists.

Subtstance P RPKPQQFFGLM-NH2
Uperolein BPDPNAFYGLM-NH2
Physalaemin BADPNKFYGLM-NH2
Eledoisin BPSKDAFIGLM-NH2
Phyllomedusin BPNPNRFIGLM-NH2

**Fig. 1.** Sequences of Physalaemin subfamily of Tachykinins.

## 2. Materials and methods

### 2.1. Materials

Phyllomedusin (BNPNRFIGLM-[$NH_2$]) was custom synthesized by Princeton biomolecules (PA, USA). The purity of peptide reported by HPLC analysis was >98%. Perdeuterated DPC ($d_{38}$) was obtained from Cambridge isotope laboratories (Massachusetts, USA). NMR reagents were obtained from Aldrich chemical company (Milwaukee, Wisconsin, USA). TFE, SDS, $CaCl_2$ were obtained from Sigma, in the highest available purity.

### 2.2. CD spectropolarimetry

CD spectra were recorded on a Jasco J-720 spectropolarimeter (Jasco, Tokyo, Japan), using a 1-mm quartz cell. The instrument was calibrated using d-10-camphorsulfonic acid (Chen and Yang, 1977). The concentration of the peptide was 114 μM. Spectra were the average of 4 scans recorded with a 1 nm bandwidth, a 0.25 nm step size, and a 0.2 s time constant between 190 and 250 nm. The molar ellipticity ([$\theta$]), expressed in deg.cm$^2$dmol$^{-1}$ was calculated from the observed ellipticity after baseline correction, using the relationship $[\theta] = \theta/lC$, where $\theta$ is the observed ellipticity, $l$ is the path length in millimeters and $C$ is the molar concentration. All the measurements were performed at the room temperature. Different solvents were used so as to mimic the different biomembrane compartments. The CD spectra for the peptide were recorded in sodium phosphate buffer, pH 7.2, increasing concentration of TFE, anionic detergent SDS and zwitterionic lipid DPC. The CD spectra for Phyllomedusin were also recorded in presence of DPC, SDS micelles and TFE and were corrected by subtracting the spectra of respective solutions. Effect of calcium ions on the conformation of the peptide was also studied using CD spectropolarimetry. For $Ca^{2+}$ titrations, aliquots of stock solution of $CaCl_2$ in TFE solutions were added to the peptide solution so as to get the molar ratio of 1:1, 2:1, 5:1, 10:1 and 20:1.

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