Structure-based differences in the membrane insertion properties of Dynorphin A(1-13) and Des-Tyr dynorphin

R. Sankararamakrishnan* and H. Weinstein, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York 10029
* sankar@inka.mssm.edu

DYNORPHIN A-(1-13) AND DES-TYR DYNORPHIN BEHAVE DIFFERENTLY IN PHOSPHOLIPID BILAYERS.
Dynorphin A-(1-17) [DynA-(1-17)] is an endogenous opioid peptide with selectivity for k-opioid receptors. The shorter DynA-(1-13) fragment has practically the same pharmacological profile. With the deletion of the first residue, the peptide DynA-(2-17) (des-Tyr-Dynorphin) does not bind to opioid receptors, but has other, non-opiate functions. The suggestion that opioid ligands-receptor interaction occurs through membrane immersion prompted our previous molecular dynamics (MD) studies of DynA-(1-17) in DMPC bilayers (*Biophys. J.* 79 (2000), in press). These revealed a tilted orientation of the peptide with respect to the bilayer normal and showed how specific residues participate in characteristic interactions resulting in the specific mode of peptide stabilization in the bilayer. New MD simulations of DynA-(1-13) and DynA-(2-17) in DMPC bilayers for 5-7 ns (after 1 ns equilibration) show that DynA-(1-13) in bilayers is oriented similarly to DynA-(1-17). In contrast, absence of the first Tyr residue in DynA-(2-17) results in deeper penetration, and a different orientation of the peptide within the bilayer. Solvation profiles, water penetration and interaction energy analysis show how Tyr is responsible for the difference in behavior between des-Tyr-Dynorphin and the DynA-(1-17)/DynA-(1-13) peptides in membranes.

Introduction

• Dynorphin A (1-17) [Dyn A(1-17)] is an endogenous opioid peptide, selective for the kappa-opioid receptors [1]. It is a potential analgesic and is believed to have low abuse potential. It has been proposed to have a characteristic interaction pattern in phospholipid bilayers [2]. The sequence of the flexible Dyn A(1-17) is given by:

Y^1 G G F L R R I R P^{10} K L K W D N Q^{17}

• The smaller fragment Dyn A(1-13) has practically the same pharmacological profile as that of its parent peptide [1].

• Des-tyrosine dynorphin [Dyn A(2-17)] does not bind to opioid receptors [3], but both dynorphin and its des-tyr fragments exhibit various non-opioid biological functions [4].

• It has been proposed that before interacting with the receptor, the peptide hormones will accumulate in the lipid bilayer and the lipid medium will induce a stable, bio-active conformation [5].

• Recent NMR structure of Dyn A(1-17) obtained in DPC micelles consisted of an alpha-helical segment (residues 3 to 9) in the N-terminal region, a beta-turn (residues 14 to 17) and a linker region (residues 10 to 13) [6].

• Based on hydrophobic labeling and spectroscopic studies, Schwyzer [4,7] suggested that the more hydrophobic N-terminal helical segment of Dyn A(1-17) will be oriented perpendicular to the membrane surface, contacting the hydrophobic membrane region whereas the extended C-terminal segment would be in contact with the aqueous phase.

• Two parallel simulations of Dyn A(1-17) in DMPC bilayers converged to the same structure in which the N-terminal helical segment of Dyn A(1-17) adopted a tilted orientation within the bilayers [2]. Analysis of the simulation studies showed that specific interactions of residues with lipids and water resulted in such orientation. For example, in both the simulations, Tyr-1 residue preferred to be close to lipid head groups and Phe-4 residue was pointing towards the center of the bilayer.

• In this work, we investigate the properties of Dyn A(1-17) fragments in the bilayers. In order to probe the role of the Tyr-1 residue, we carried out multi-nano second molecular dynamics simulations on Dyn A(2-17) and Dyn A(1-13) peptides in DMPC bilayers.

Methods

• NVE ensemble

• Dyn A(1-13) and Dyn A(2-17) structures were constructed from the NMR internal parameters [6]. The N-terminal helix was placed inside the DMPC bilayers, oriented perpendicular to the membrane as suggested by Schwyzer [5,7]. The C-terminal region lied approximately parallel to the membrane plane.

• Z = 0 Å was the center of the bilayer and the Z-axis was the bilayer normal. Dynorphin helical segment was placed at Z = 10 Å.

• The protocol developed by Woolf and Roux [8] was used to construct the peptide - hydrated lipid system.

• In the bilayer, the top layer contains 41 lipids and the peptide. The bottom layer is composed of 45 lipids.
~ 5,300 waters; total: ~26,000 atoms - Dyn A(2-17)
~ 2,600 waters; total: ~18,000 atoms - Dyn A(1-13)

• Other simulation details:
Temperature: 330 K; Time step: 0.002 ps.
Equilibration 1.0 to 1.5 nanoseconds
Production run 5 ns for Dyn A(2-17) and 8 ns for Dyn A(1-13).

Results and Discussion

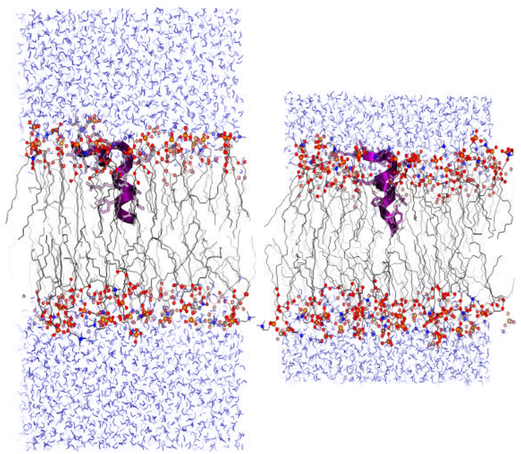

Figure 1: Starting structures of Dyn A(2-17) (left) and Dyn A(1-13) (right) in DMPC bilayers. Larger number of water molecules were included for Dyn A(2-17) system to solvate the longer C-terminal segment, as in our previous simulations on Dyn A(1-17) [2]. The following color code system is used: water: blue, peptide: pink, choline nitrogen: dark blue, phosphorous: orange and lipid carbonyl oxygens: red.

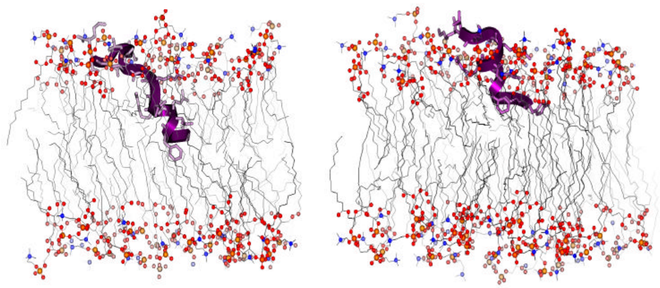

Figure 2: Final structures and orientations at the end of 5 ns (Dyn A(2-17) - left) and 8 ns (Dyn A(1-13) - right) production runs. The N-terminal helical segment remains imbedded within the bilayers in both simulations. However, while Dyn A(1-13) adopts similar orientation as that of Dyn A(1-17) [2], the helical segment of Dyn A(2-17) penetrates deeper into the bilayer.

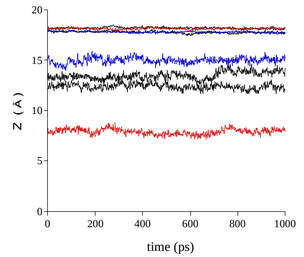

Figure 3: Molecular Dynamics trajectory of the center of mass location of helical segments along the bilayer normal. Dyn A(2-17) helix (red) has moved 7 Å deeper into the bilayer compared to Dyn A(1-13) (blue). For comparison purpose, the positions of Dyn A(1-17) helical segments (black) from our previous simulations [2] are also shown. The position of Dyn A(1-13) helix is closer to Dyn A(1-17). The dotted curves represent the average positions of lipid phosphorous and nitrogen atoms. Analysis was carried out for the last 1 ns of the production run.

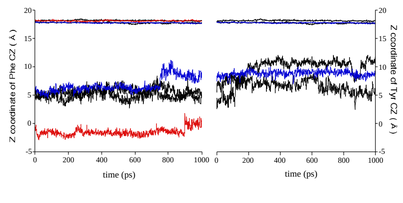

Figure 4: Center of mass location along the bilayer normal for the CZ atoms of Phe and Tyr residues. The absence of first tyrosine residue in Dyn A(2-17) (red) allows the Phe residue to go beyond the center of the bilayer. Although the C-alpha of Phe is closer to the lipid head group than C-alpha of Tyr, the aromatic side chains point in the opposite directions in Dyn A(1-13) (blue). A similar behavior is observed in Dyn A(1-17) (black) simulations [2]. In all these simulations, Phe prefers a much hydrophobic center of the bilayer and Tyr moves closer to the lipid head groups. For other details, see Figure 3.

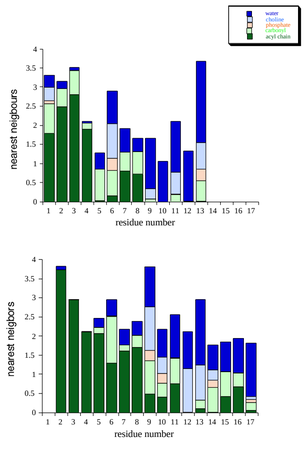

Figure 5: Average number of water molecules, acyl chain carbon atoms and lipid head groups surrounding each side chain for Dyn A(1-13) (above) and Dyn A(2-17) (below). As in Dyn A(1-17) [2], the basic residues participate in a "snorkel model" type interactions [9] in both simulations. The non-polar part of the long arginine side chains is surrounded by lipid hydrocarbon and the positively charged guanidinium group is exposed to water. In Dyn A(2-17), the first four N-terminal residues interact predominantly with the acyl chains. In Dyn A(1-13), in addition to the above interactions, these N-terminal residues and tyrosine also interact significantly with the phospholipid head groups and water. To make the visual comparison easier, the numbering of amino acids in Dyn A(2-17) begins from 2.

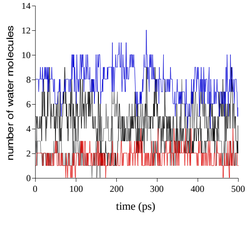

Figure 6: MD trajectories of the number of water molecules that are within 5 Å from the first four residues (Dyn A(2-13)-blue, Dyn A(1-17)-black [7]) or first three residues (Dyn A(2-17)-red) in the N-terminus. The absence of tyrosine resulted in a small number of waters penetrating near the N-terminus for Dyn A(2-17). This number in Dyn A(1-13) (and Dyn A(1-17)) is almost three times larger than that in Dyn A(2-17). The orientation and depth of the helical segment with respect to the membrane-water interface influence the water penetration near the N-terminus. Analysis was carried out for the last 500 ps of production run.

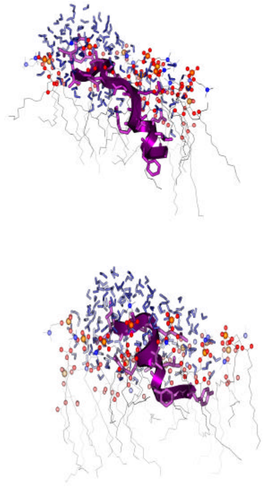

Figure 7: Water molecules within 5 Å from the peptide are plotted along with dynorphin peptides. In Dyn A(2-17) (top), less water penetration is observed near the N-terminus. The features of water penetration in Dyn A(1-13) (bottom) are similar to Dyn A(1-17) simulations [2]. Also shown are the lipids from the top layer that make at least one contact with the peptide within 5 Å.

Summary and Conclusions

• Dyn A(1-13) and Dyn A(2-17), the opioid and non-opioid fragments of dynorphin were studied in DMPC bilayers with the multi-nanosecond molecular dynamics simulations.

• The N-terminal helical segments of both the peptides were initially inserted in a similar manner within the bilayer; the helical segments were oriented perpendicularly with respect to the membrane plane, at the same heights.

• As observed in the parent peptide Dyn A(1-17) [2], the helical segments remained stable within the bilayers in both simulations.

• In Dyn A(2-17), the N-terminal segment went deeper inside the bilayers by more than 7 Å in comparison to Dyn A(1-13). The position of Dyn A(1-13) helix was similar to Dyn A(1-17) observed in our previous simulations [2].

• While the Phe was observed to be in a hydrophobic environment in both simulations, the tyrosine residue in Dyn A(1-13) preferred to be close to the lipid head group and water environment. This agrees with experimental observations [10] suggesting that Tyr/Trp and Phe have a different preference for the locations within the lipid bilayers, attributable to the difference in chemical properties of the side chains.

• Arginine residues contribute significantly towards the peptide-lipid and peptide-water interactions.

• The absence of Tyr results in less water penetration near the first few N-terminal residues in Dyn A(2-17). The water penetration in Dyn A(1-13) is similar to that of Dyn A(1-17) [2].

• The mechanistic role of Tyr-1 in keeping the peptide close to the membrane-water interface through specialized interactions, as observed in Dyn A(1-13)/Dyn A(1-17) simulations, is likely to be a determinant factor for the binding mechanism of dynorphin with the opioid receptor.

References

[1] Chavkin, C. and Goldstein, A. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6543-6547 (1981).

[2] Sankararamakrishnan, R. and Weinstein, H. *Biophys. J.* **79**, 2331-2344 (2000)

[3] Walker, J.M., Moises, H.C., Coy, D.H., Baldrighi, G. and Akil, H. *Science* **218**, 1136-1138 (1982).

[4] Shukla, V.K. and Lemaire S. *TIPS* **15**, 420-424 (1994).

[5] Schwyzer, R. *Biopolymers* **37**, 5-16 (1995).

[6] Tessmer, M.R. and Kallick, D.A. *Biochemistry* **36**, 1971-1981 (1997).

[7] Erne, D., Sargent, D.F. and Schwyzer, R. *Biochemistry* **24**, 4261-4263 (1985).

[8] Woolf, T.B. and Roux, B. *Proteins* **24**, 92-114 (1996).

[9] Segrest, J.P., DeLoof, H., Dohlman, J.G., Brouillette, C.G. and Anantharamaiah, G.M. *Proteins* **8**, 103-117 (1990).

[10] Killian, J.A. and von Heijne, G. *TIBS* **25**, 429-434 (2000).

Acknowledgements

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