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PII: S0005-2736(17)30044-5
DOI: doi:10.1016/j.bbamem.2017.01.036
Reference: BBAMEM 82418

To appear in: *BBA - Biomembranes*

Received date: 28 November 2016
Revised date: 16 January 2017
Accepted date: 28 January 2017

Please cite this article as: Xiaochao Chen, Shutao Liu, Bruno Deme, Viviana Cristiglio, Drew Marquardt, Richard Weller, Pingfan Rao, Yunqiang Wang, Jeremy Bradshaw, Efficient internalization of TAT peptide in zwitterionic DOPC phospholipid membrane revealed by neutron diffraction, *BBA - Biomembranes* (2017), doi:10.1016/j.bbamem.2017.01.036

Efficient internalization of TAT peptide in zwitterionic DOPC phospholipid membrane revealed by neutron diffraction

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Abstract

The aim of this study is to investigate the interactions between TAT peptides and a neutral DOPC bilayer by using neutron lamellar diffraction. The distribution of TAT peptides and the perturbation of water distribution across the DOPC bilayer were revealed. When compared to our previous study on an anionic DOPC/DOPS bilayer (X. Chen *et al*., Biochim Biophys Acta. 2013. 1828 (8), 1982-1988), a much deeper insertion of TAT peptides was found in the hydrophobic core of DOPC bilayer at a depth of 6.0 Å from the center of the bilayer, a position close to the double bond of fatty acyl chain. We conclude that the electrostatic attractions between the positively charged TAT peptides and the negatively charged headgroups of phospholipid are not essential for the direct translocation. Furthermore, the interactions of TAT peptides with the DOPC bilayer were found to vary in a concentration-dependent manner. A limited number of peptides first associate with the phosphate moieties on the lipid headgroups by using the guanidinium ions pairing. Then the energetically favorable water defect structures are adopted to maintain the arginine residues hydrated by drawing water molecules and lipid headgroups into the bilayer core. Such bilayer deformations consequently lead to the deep intercalation of TAT peptides into the bilayer core. Once a threshold concentration of TAT peptide in the bilayer is reached, a significant rearrangement of bilayer will happen and steady-state water pores will form.

Keywords: cell penetrating peptide; TAT peptide; neutron diffraction; phospholipid

Introduction

Since the first discovery of HIV-TAT peptide in late 1980s, a large family of short peptides capable of penetrating cell membrane without causing significant membrane defect have been specifically termed as cell penetrating peptides (CPPs) [1,2]. They are very promising transporters for intracellular delivery of various bioactive molecules with high efficiency, low cytotoxicity and low immunogenicity [3-9]. Numerous experimental studies have been established to better understand how CPPs manage to overcome the prodigious thermodynamic cost of membrane internalization [10-12]. However, the detailed mechanisms of internalization of CPPs are still controversial which depend on a great variety of aspects such as the nature and size of CPP and its cargo, the concentration used, the temperature involved, and the cell lines targeted etc [13-15]. Despite of a lot of controversy and debate, the common consensus is that both direct translocation and energy-dependent endocytosis