Chemistry and Physics of Lipids 196 (2016) 33–51

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Maximally asymmetric transbilayer distribution of anionic lipids alters the structure and interaction with lipids of an amyloidogenic protein dimer bound to the membrane surface

Sara Y. Cheng[a,*], George Chou[b], Creighton Buie[b], Mark W. Vaughn[b], Campbell Compton[c], Kwan H. Cheng[d,e,**]

[a] Department of Physics, University of Texas at Austin, Austin, TX 78712, USA
[b] Department of Chemical Engineering, Texas Tech University, Lubbock, TX 79409, USA
[c] Department of Computer Science, Trinity University, San Antonio, TX 78212, USA
[d] Department of Physics, Texas Tech University, Lubbock, TX 79409, USA
[e] Department of Physics and Astronomy, Trinity University, San Antonio, TX 78212, USA

ARTICLE INFO

Article history:
Received 16 August 2015
Received in revised form 13 January 2016
Accepted 14 January 2016
Available online 28 January 2016

Keywords:
Molecular dynamics simulations
Asymmetric lipid membrane
Protein structures on surfaces
Protein–lipid interactions
Protein aggregation
Beta-amyloid

ABSTRACT

We used molecular dynamics simulations to explore the effects of asymmetric transbilayer distribution of anionic phosphatidylserine (PS) lipids on the structure of a protein on the membrane surface and subsequent protein–lipid interactions. Our simulation systems consisted of an amyloidogenic, beta-sheet rich dimeric protein (D42) absorbed to the phosphatidylcholine (PC) leaflet, or protein-contact PC leaflet, of two membrane systems: a single-component PC bilayer and double PC/PS bilayers. The latter comprised of a stable but asymmetric transbilayer distribution of PS in the presence of counterions, with a 1-component PC leaflet coupled to a 1-component PS leaflet in each bilayer. The maximally asymmetric PC/PS bilayer had a non-zero transmembrane potential (TMP) difference and higher lipid order packing, whereas the symmetric PC bilayer had a zero TMP difference and lower lipid order packing under physiologically relevant conditions. Analysis of the adsorbed protein structures revealed weaker protein binding, more folding in the *N*-terminal domain, more aggregation of the *N*- and *C*-terminal domains and larger tilt angle of D42 on the PC leaflet surface of the PC/PS bilayer versus the PC bilayer. Also, analysis of protein-induced membrane structural disruption revealed more localized bilayer thinning in the PC/PS versus PC bilayer. Although the electric field profile in the non-protein-contact PS leaflet of the PC/PS bilayer differed significantly from that in the non-protein-contact PC leaflet of the PC bilayer, no significant difference in the electric field profile in the protein-contact PC leaflet of either bilayer was evident. We speculate that lipid packing has a larger effect on the surface adsorbed protein structure than the electric field for a maximally asymmetric PC/PS bilayer. Our results support the mechanism that the higher lipid packing in a lipid leaflet promotes stronger protein–protein but weaker protein–lipid interactions for a dimeric protein on membrane surfaces.

1. Introduction

Protein structural transitions on cell membrane surfaces are important molecular events in regulating normal and pathogenic cellular processes (Gething and Sambrook, 1992; Bucciantini and Cecchi, 2010). Upon binding to a surface, a protein undergoes surface-induced and localized secondary structural changes, such as folding or unfolding of ordered helices or beta-sheets in different domains. On the membrane surface, these folded or unfolded domains may further interact and undergo complex higher order tertiary and quaternary structural changes, hydrodynamic shape alterations, and intra- or inter-peptide domain aggregations (Vymetal and Vondrasek, 2011; Stefani, 2012). Membrane-orientational transitions of protein, such as tilted or non-tilted conformation of certain motifs, may also occur (Basyn et al., 2001) on the surface. All of these altered protein structures may provide the necessary functional motifs for receptor-based binding, such as cell signaling and immunological responses

* Corresponding author.
** Corresponding author at: Department of Physics and Astronomy, Trinity University, San Antonio, Texas 78212, USA.
E-mail addresses: sara.y.cheng@utexas.edu (S.Y. Cheng), kcheng1@trinity.edu (K.H. Cheng).

http://dx.doi.org/10.1016/j.chemphyslip.2016.01.002
0009-3084/

(Martin and Hartl, 1997; Kurre et al., 2001; Kanekiyo et al., 2014). For an intrinsically disordered and aggregation-prone, or amyloidogenic, protein, the altered protein structure on the surface may trigger irreversible self-aggregation in several membrane-associated cytotoxic aggregation cascade pathways, e.g., beta-amyloid cascade in Alzheimer's pathogenesis (Ross and Poirier, 2004; Ellis, 2006). At present, the role of transbilayer lipid distribution in regulating the above protein transitional events of the amyloidogenic protein is unclear.

It is commonly thought that lipids are not randomly distributed but form compositionally distinct domains along the normal of the planar lipid membrane. These lipid domains are commonly known as asymmetric transbilayer lipid domains (van Meer et al., 2008). Various asymmetric transbilayer lipid distributions, resulting from two different kinds of lipids in a bilayer, can elicit very different properties than those of symmetric one-component lipid distribution. Examples of asymmetric lipid distributions include zwitterionic phosphatidylcholine (PC) lipids in one leaflet and charged, anionic or cationic, lipids in the other leaflet. Such lipid arrangements represent the maximally asymmetric transbilayer distribution possible in a biological membrane. This highly asymmetric lipid arrangement may have different lipid order packing and transmembrane potential distribution (Gurtovenko and Vattulainen, 2008) when compared with the symmetric bilayer. Among the various key lipids types, anionic phosphatidylserine (PS) is of interest because of its distinct roles in cell surface signaling. PS can trigger apoptosis, protein trafficking, and immunological responses, in various cells (Williamson and Schlegel, 1994; Fadok et al., 2001; Yeung et al., 2008; Fadeel and Xue, 2009; Lingwood and Simons, 2010). Here, two bilayer systems: an asymmetric double bilayer PC/PS bilayer system consisting of a PC monolayer coupled to a PS monolayer in each bilayer and a symmetric, one-component PC bilayer control, were investigated.

For the maximally asymmetric PC/PS double bilayer system, the asymmetric charge arrangement of the PS lipids in the presence of counterions has been shown to create a non-zero transmembrane potential (TMP) difference of approximately −240 mV measured from the PS side towards the PC side of the bilayer (Gurtovenko and Vattulainen, 2008). This is the same sign as the local TMP difference found in cells. In contrast, no TMP difference occurs in the control PC bilayer system. Interestingly, the large difference in the melting temperatures of PC and PS lipids in one-component bilayers implies a higher order packing of the PC monolayer in the highly coupled PC/PS bilayer than in the PC bilayer. How the TMP and order packing lipid properties affect the structures of an amyloidogenic protein dimer, a simplest model multimer, is unclear.

A beta-sheet rich beta-amyloid dimer (Luhrs et al., 2005) was chosen as our model amyloidogenic protein multimer in this study. Recent atomic force microscopy studies (Jang et al., 2010; Jang et al., 2013) have indicated that amyloidogenic beta-amyloid multimers exhibit beta-sheet rich fibril morphology. This fibril structure has a U-shaped beta-strand-loop-beta-strand motif with an exposed hydrophobic surface in the C-terminal domain that interacts with the cell membranes. Recent molecular dynamics (MD) simulations (Jang et al., 2010; Poojari and Strodel, 2013; Nasica-Labouze et al., 2015) further established the capability of these fibrillar beta-amyloid multimers to form pores in a simple one-component lipid bilayer. At present, the knowledge of early events of protein structural transitions and protein–lipid interactions of any beta-amyloid multimers on asymmetric lipid surfaces that may lead to membrane pore formation is unclear.

We used molecular dynamics (MD) simulations to investigate the effects of asymmetric transbilayer lipid distribution on the structure of D42 on the membrane surface of PC/PS and PC bilayers. In addition, the effects of the absorbed protein on the bilayer structure of the PC/PS and PC systems were compared. Our goal was to elucidate the lipid–protein interaction mechanisms of how the transbilayer lipid asymmetry regulates the complex protein structural transitions and the membrane structural disruption behaviors of an amyloidogenic protein on membrane surfaces that may lead to membrane pore formation.

2. Materials and methods

2.1. Starting structures

2.1.1. Protein

A beta-sheet rich, fibril-like beta-amyloid dimer (D42) was used. This protein has two chains (A and B), both with 42 residues and identical sequence (Hardy and Higgins, 1992) as given below.

H–ASP–ALA–GLU–PHE–(ARG-5)–HIS–SER–GLY–TYR–GLU–VAL–HIS–HIS–GLN–(LYS-16)–LEU–**VAL–PHE–PHE–ALA–GLU–ASP–VAL–GLY–SER–ASN–(LYS-28)–GLY–ALA–ILE–ILE–GLY–LEU–MET–VAL–GLY–GLY–VAL–VAL–ILE–ALA–OH**

At neutral pH, the sequence has 7 negative charges (ASP-1, GLU-3, ASP-7, GLU-11, GLU-22, ASP-23 and *C*-terminal end carboxylate) and 4 positive charges (*N*-terminal end amine, ARG-5, LYS-16 and LYS-28). Hence, D42 (chain A and chain B) has a net charge of −6. Each chain has a distinct *N*-terminal domain and a *C*-terminal domain. Therefore, D42 has a total of four structural domains: *N*-terminal domain of chain A, *N*-terminal domain of chain B, *C*-terminal domain of chain A, and *C*-terminal domain of chain B. As shown in Fig. 1A, each *N*-terminal domain has an unstructured peptide fragment (residues 1–17) exhibiting random coil structure. In contrast, each *C*-terminal domain has a more structured U-shaped motif (residues 18–42) with a beta-strand-loop-beta-strand-coil structure, and its sequence is highlighted above in bold.

To construct the D42 dimer, the atomic coordinates of the *C*-terminal domains were extracted from the experimental NMR fibrillar structure (PDB file: 2BEG) of a beta-amyloid pentamer

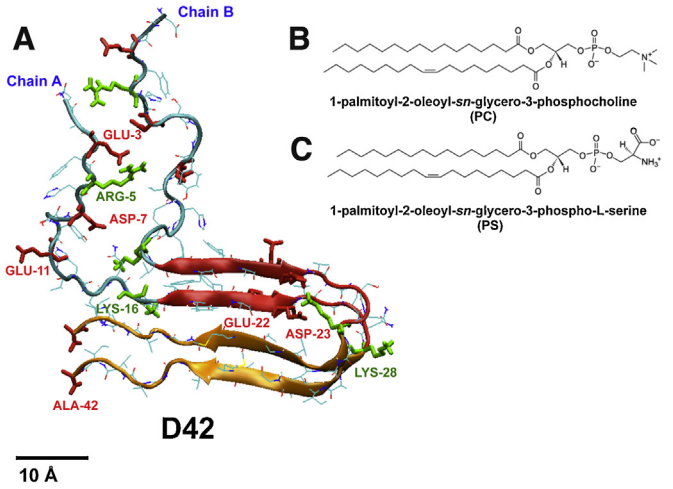

Fig. 1. Structures of protein and lipids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) The initial protein structure of D42 (A) consists of two 42-residue long peptide chains, chain A and chain B, with identical sequence (see Section 2). Each chain has a random-coil *N*-terminal domain (residues 1–17) and an U-shaped *C*-terminal domain (residues 18–42). The latter contains a compact beta-strand-to-loop-to-beta-strand-random coil (residues 18–28 in red and residues 29–42 in orange) motif. Four positively charged residues (*N*-terminal end amine, ARG-5, LYS-16 and LYS-28) and seven negatively charged residues (ASP–1, GLU–3, ASP–7, GLU–11, GLU–22, ASP–23 and *C*-terminal end carboxylate) in each chain are rendered in thick green and red licorice representations, respectively, and the non-charged residues are in thin color lines. A scale bar of 10 Å is shown. The chemical structures of the lipids, PC (B) and PS (C), are given. Here PC is zwitterionic and PS anionic, according to the charge distribution in the polar headgroup of each lipid.