Chemistry and Physics of Lipids 169 (2013) 72–84

Contents lists available at SciVerse ScienceDirect

Chemistry and Physics of Lipids

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

Detergent-mediated protein aggregation

Chris Neale[a,b], Hamed Ghanei[c], John Holyoake[a,d], Russell E. Bishop[e], Gilbert G. Privé[b,c,d], Régis Pomès[a,b,*]

[a] Molecular Structure and Function, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada
[b] Department of Biochemistry, University of Toronto, 101 College Street, Toronto, Ontario M5G 1L7, Canada
[c] Department of Medical Biophysics, University of Toronto, 101 College Street, Toronto, Ontario M5G 1L7, Canada
[d] Ontario Cancer Institute and Campbell Family Cancer Research Institute, UHN, 101 College Street, Toronto, Ontario M5G 1L7, Canada
[e] Department of Biochemistry and Biomedical Sciences and Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

ARTICLE INFO

Article history:
Available online 4 March 2013

Keywords:
Molecular dynamics
Simulation
Membrane protein
Dynamic light scattering

ABSTRACT

Because detergents are commonly used to solvate membrane proteins for structural evaluation, much attention has been devoted to assessing the conformational bias imparted by detergent micelles in comparison to the native environment of the lipid bilayer. Here, we conduct six 500-ns simulations of a system with >600,000 atoms to investigate the spontaneous self assembly of dodecylphosphocholine detergent around multiple molecules of the integral membrane protein PagP. This detergent formed equatorial micelles in which acyl chains surround the protein's hydrophobic belt, confirming existing models of the detergent solvation of membrane proteins. In addition, unexpectedly, the extracellular and periplasmic apical surfaces of PagP interacted with the headgroups of detergents in other micelles 85 and 60% of the time, respectively, forming complexes that were stable for hundreds of nanoseconds. In some cases, an apical surface of one molecule of PagP interacted with an equatorial micelle surrounding another molecule of PagP. In other cases, the apical surfaces of two molecules of PagP simultaneously bound a neat detergent micelle. In these ways, detergents mediated the non-specific aggregation of folded PagP. These simulation results are consistent with dynamic light scattering experiments, which show that, at detergent concentrations ≥600 mM, PagP induces the formation of large scattering species that are likely to contain many copies of the PagP protein. Together, these simulation and experimental results point to a potentially generic mechanism of detergent-mediated protein aggregation.

1. Introduction

All cells and their compartments are surrounded by lipid membranes to which embedded proteins bestow biological activity. These membrane proteins are crucial for signal transduction (Cantrell, 1996; Schlessinger, 2000; Wettschureck and Offermanns, 2005; Bezanilla, 2008; Gilman, 1987), molecular transport (Pohorille et al., 2005; Ackerman and Clapham, 1997; Gould and Holman, 1993), catalysis (Bishop, 2005; Zhou et al., 2008), as well as membrane fusion (Ungar and Hughson, 2003) and biosynthesis (Bishop et al., 2000). Accordingly, they are implicated in a wide variety of diseases (Sanders and Myers, 2004) and are the targets of a large portion of approved drugs (Yildirim et al., 2007).

Although integral membrane proteins adopt a variety of topologies (Bowie, 2005; von Heijne, 2006; Popot and Engelman, 2000), they are all anchored in the membrane by apolar transmembrane segments and polar flanking regions (von Heijne, 2006; Segrest and Feldmann, 1974; von Heijne, 1989; Eisenberg et al., 1984). The clustering of apolar and polar/charged residues on the surface of integral membrane proteins complements the transmembrane profile of partial charge density in their native environment (Nagle et al., 1996; Nagle and Tristram-Nagle, 2000): integral membrane proteins expose a belt of hydrophobic residues to the apolar bilayer core, and expose hydrophilic apical surfaces to zwitterionic/ionic lipid headgroups and aqueous solution (Schulz, 2002; Sipos and Von Heijne, 1993).

Three-dimensional structures offer invaluable insight into the molecular basis of protein function (Ahn et al., 2004; Hwang et al., 2002, 2004; Cuesta-Seijo et al., 2010) and provide a starting point for the rational design or enhancement of drugs that bind membrane proteins and alter their activities (Congreve and Marshall, 2010; Wacker et al., 2010; Giacomini et al., 2010; Durdagi et al., 2011). Unfortunately, the two most commonly used methods of

* Corresponding author at: Régis Pomès, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada. Tel.: +1 416 813 5686; fax: +1 416 813 5022.
E-mail address: pomes@sickkids.ca (R. Pomès).

0009-3084/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemphyslip.2013.02.005

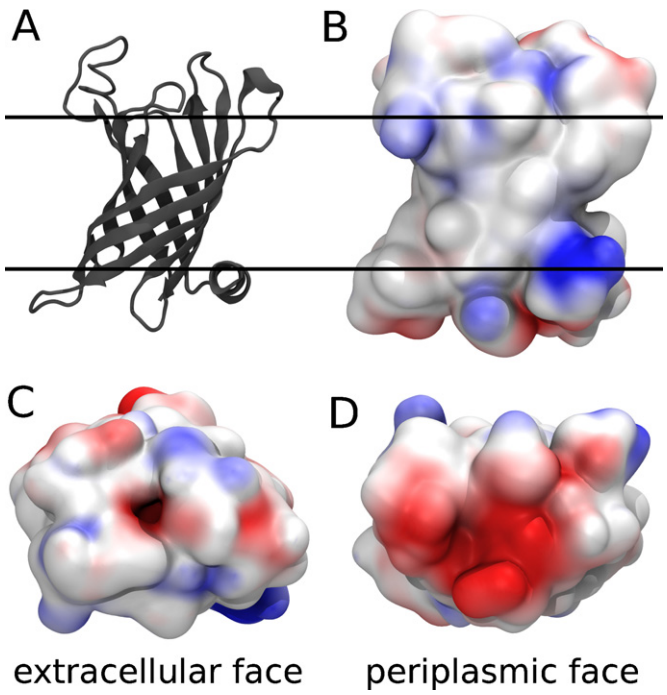

Fig. 1. The Gram negative bacterial outer membrane enzyme PagP. (A) Cartoon representation of the crystal structure of PagP in LDAO (PDB ID 1THQ) (Ahn et al., 2004). The proposed (Ahn et al., 2004) bilayer interfaces are depicted as solid lines. (B–D) Electrostatic potential of PagP, computed by the adaptive Poisson-Boltzmann solver (Baker et al., 2001), mapped onto its van der Waals surface by VMD (Humphrey et al., 1996) showing (B) side view along the proposed (Ahn et al., 2004) bilayer plane, and (C) extracellular and (D) periplasmic apical surfaces. (Red) Electronegative and (blue) electropositive regions of the protein surface are highlighted. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

high-resolution structural determination, solution NMR and X-ray crystallography, are not well suited to study proteins embedded in lipid bilayers. One the one hand, the large size of lipid bilayers leads to long rotational correlation times and therefore low signal resolution in solution NMR (Opella et al., 2002). On the other hand, target protein concentrations in lipid bilayers are usually low, presenting a bottleneck to the production of high-quality crystals (Schulz, 2002; Bill et al., 2011). These problems can be circumvented by replacing the lipid membrane with a mimetic whose properties are more suitable to the experimental approach. A membrane mimetic is necessary because the structure of a membrane protein depends on its environment (White et al., 2001; White and Wimley, 1999) and the native tertiary and/or quaternary structure is therefore not generally maintained in aqueous solution.

One popular membrane mimetic strategy is to replace lipids with detergents, which can solvate membrane proteins by forming expanded micelles around their hydrophobic belts (Tamm and Liang, 2006). For example, the β-barrel Gram-negative bacterial outer membrane enzyme PagP (Bishop, 2005) (Fig. 1) adopts similar folds when solvated by detergents such as dodecylphosphocholine (DPC) (Hwang et al., 2002; Hwang and Kay, 2005), lauryldimethylamineoxide (LDAO) (Ahn et al., 2004; Khan et al., 2007), CYFOS-7 (Hwang et al., 2004; Hwang and Kay, 2005), *n*-octyl-β-D-glucoside (OG) (Hwang et al., 2002; Hwang and Kay, 2005), and a mixture of sodium dodecylsulfate (SDS) and 2-methyl-2,4-pentanediol (MPD) (Cuesta-Seijo et al., 2010). Furthermore, the specific activity of PagP after denaturation, purification, and refolding into DPC and CYFOS-7 micelles is indistinguishable (Hwang et al., 2002) from that of native PagP purified from membranes (Bishop et al., 2000), indicating that the conserved fold of PagP observed in complexation with detergents is a native state.

PagP is an enzyme of lipid metabolism whose role is to catalyze the transfer of a palmitate group from the *sn*-1 position of a phospholipid to the N-linked hydroxymyristate on the proximal unit of lipid A (Bishop, 2005; Bishop et al., 2000). This modification protects the Gram-negative bacterial outer membrane against disruption by cationic antimicrobial peptides and thus promotes intracellular infection and virulence (Robey et al., 2001). The binding pocket for the donor acyl chain is a deep hydrophobic furrow in the extracellular face of PagP, which acts as a hydrocarbon ruler to select 16-carbon chains for the acylation reaction (Ahn et al., 2004; Khan et al., 2007). As such, detergents that mimic fatty acids can interfere with PagP's activity by binding to the interior palmitate recognition pocket. Indeed, a detergent molecule was observed deep inside the hydrocarbon ruler in crystal structures of PagP obtained in LDAO (Ahn et al., 2004) and SDS/MPD (Cuesta-Seijo et al., 2010). Specific activity is therefore measured using dodecylmaltoside (DDM) (Cuesta-Seijo et al., 2010; Khan et al., 2010) or CYFOS-7 (Hwang et al., 2004), which possess bulky tail groups that exclude them from the hydrocarbon ruler.

Although there are now multiple crystallographic (Ahn et al., 2004; Cuesta-Seijo et al., 2010) and NMR (Hwang et al., 2002, 2004) structures of PagP, it is generally difficult to obtain detergent–protein complexes that are stable and monodisperse, and in which the protein is correctly folded (Bill et al., 2011; Privé, 2007). This difficulty is in part due to the propensity of membrane proteins to aggregate in solution.

The non-specific aggregation of proteins in solution with detergents is generally thought to be mediated by interactions between protein surfaces that are exposed upon protein unfolding (Privé, 2007). However, there are no experimentally-derived structures of disordered aggregates because the formation of such aggregates precludes their high-resolution experimental evaluation. Even experimental methods that primarily produce medium- and low-resolution structural representations, such as cryo-electron microscopy and small angle X-ray or neutron scattering, are poorly suited to evaluate the conformational preferences of heterogeneous aggregates because these methods provide ensemble-averaged structural depictions that cannot be deconvoluted.

To rationally facilitate the solvation of membrane proteins for experimental structure determination, we must first understand the interactions between membrane proteins and the detergents and/or lipids that are used for their solubilization. In this perspective, interactions between proteins and detergents or lipids are revealed in some high-resolution X-ray structures (Lee, 2003) and have been investigated by NMR (Fernández et al., 2002; Hilty et al., 2004; Roosild et al., 2005; Chill et al., 2006; Lee et al., 2008). To complement these experimental approaches, computer simulations can provide atomistic details of the conformational preferences of both soluble and insoluble species as well as direct insight to the mechanisms of solvation and aggregation.

The first computer simulation of a peptide in a detergent micelle was published in 1999 (Wymore and Wong, 1999). Since then, simulations have been used to characterize the conformations and interactions of many other proteins and peptides in various detergents. While most of these studies have been initiated with a preformed detergent micelle (Wymore and Wong, 1999; Rodríguez-Ropero and Fioroni, 2012; Friemann et al., 2009; Cuthbertson et al., 2006; Lagüe et al., 2005; Khandelia and Kaznessis, 2005a,b; Löw et al., 2008; Langham et al., 2007; Chevalier et al., 2006; Patargias et al., 2005; Bond and Sansom, 2003; Khao et al., 2011; Renthal et al., 2011; Cox and Sansom, 2009; Choutko et al., 2011; Krishnamani and Lanyi, 2012; Sands et al., 2006; Psachoulia et al., 2006), simulations have also evaluated the self-assembly of detergents around a protein or peptide using atomistic (Psachoulia et al., 2006; Bond et al., 2004; Böckmann and Caflisch,