Biochimica et Biophysica Acta 1828 (2013) 2592–2608

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Structural adaptations of proteins to different biological membranes

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ARTICLE INFO

Article history:
Received 29 March 2013
Received in revised form 4 June 2013
Accepted 19 June 2013
Available online 27 June 2013

Keywords:
Lipid bilayer
Membrane protein
Hydrophobic thickness
Polarity profile
Membrane asymmetry
Hyperthermophile

ABSTRACT

To gain insight into adaptations of proteins to their membranes, intrinsic hydrophobic thicknesses, distributions of different chemical groups and profiles of hydrogen-bonding capacities (α and β) and the dipolarity/polarizability parameter (π*) were calculated for lipid-facing surfaces of 460 integral α-helical, β-barrel and peripheral proteins from eight types of biomembranes. For comparison, polarity profiles were also calculated for ten artificial lipid bilayers that have been previously studied by neutron and X-ray scattering. Estimated hydrophobic thicknesses are 30–31 Å for proteins from endoplasmic reticulum, thylakoid, and various bacterial plasma membranes, but differ for proteins from outer bacterial, inner mitochondrial and eukaryotic plasma membranes (23.9, 28.6 and 33.5 Å, respectively). Protein and lipid polarity parameters abruptly change in the lipid carbonyl zone that matches the calculated hydrophobic boundaries. Maxima of positively charged protein groups correspond to the location of lipid phosphates at 20–22 Å distances from the membrane center. Locations of Tyr atoms coincide with hydrophobic boundaries, while distributions maxima of Trp rings are shifted by 3–4 Å toward the membrane center. Distributions of Trp atoms indicate the presence of two 5–8 Å-wide midpolar regions with intermediate π* values within the hydrocarbon core, whose size and symmetry depend on the lipid composition of membrane leaflets. Midpolar regions are especially asymmetric in outer bacterial membranes and cell membranes of mesophilic but not hyperthermophilic archaebacteria, indicating the larger width of the central nonpolar region in the later case. In artificial lipid bilayers, midpolar regions are observed up to the level of acyl chain double bonds.

1. Introduction[2]

Biological membranes provide a functional platform for integral transmembrane (TM) proteins and more temporarily bound peripheral proteins and peptides. Integral membrane proteins constitute a large part of biological membranes ranging from 20% to 80% by mass. They play important roles in vital biological processes including protein synthesis, trafficking, ionic conductance, electron and molecular transport, signal transduction, cell adhesion, cell communication, immune response, respiration, and energy metabolism.

The unique feature of membrane proteins is that they evolve and function in the highly anisotropic lipid environment. Physical and chemical properties of the lipid bilayer are essential for protein structure, functional dynamics, spatial localization and interactions with other proteins and small molecules [1–4]. In particular, the stability of protein complexes is defined by the strength of hydrogen bonds, hydrophobic, electrostatic, and van der Waals forces [5,6], which depend on the local dielectric environment of protein atoms and, therefore, on spatial arrangement of proteins in membranes [7,8].

To ensure solubility of proteins in membranes, polarity of the lipidic phase should match the polarity of embedded proteins. To maintain the functionally required degree of structural flexibility of proteins, the membrane fluidity should be strictly regulated in different cells and in different environmental conditions by adjusting the lipid composition [9]. In addition, the presence of certain lipid species at distinct locations in membranes is essential for proper membrane protein folding, sorting, targeting, and functioning [10–12]. Therefore, maintenance and regulation of compositional diversity of lipids consume a considerable amount of ATP and require proteins encoded by up to 5% of the genome [13].

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[2] Abbreviations: *DEPC*, 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (diC22:1PC); DHPC, 1,2-di-O-hexadecyl-sn-glycero-3-phosphocholine (diC16:0ePC,); DLPC, 1,2-dilauroyl-*sn*-glicero-3-phosphatidylcholine (diC12:0PC); DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (diC14:0PC); DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (di1C18:1PC); DPhyPC, 1,2-di-(3,7,11,15-tetramethylhexadecanoyl)-*sn*-glycero-3-phosphocholine (di(16:0(3me, 7me, 11me, 15me)PC); DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (diC16:0PC); ER, endoplasmic reticulum; IM, inner membrane; LPS, lipopolysaccharide; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; OM, bacterial outer membrane; OPM, Orientations of Proteins in Membranes (database); PI, liver L-α-phosphatidylinositol; PM, plasma membrane; POPG, 1-palmitoyl-2-oleoyl-*sn*-glicero-3-phosphatidylglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glicero-3-phosphatidylcholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glicero-3-phosphatidylethanolamine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glicero-3-phosphatidylserine; PPM, Positioning of Proteins in Membranes (method); SM, egg sphingomyelin; TM, transmembrane.

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http://dx.doi.org/10.1016/j.bbamem.2013.06.023

TM α-helices, β-barrels, and β-helices are the only known protein folds that fulfill the requirement to saturate the hydrogen bonding potential of the polypeptide main chain in the hydrophobic environment. TM α-helical proteins are highly abundant in all types of cellular and intracellular membranes and are encoded by ~25–30% of genes of all sequenced organisms [14]. In contrast, the TM β-barrels are mostly found in outer membranes of bacteria, mitochondria and chloroplasts, and are estimated to be encoded by less than 3% of bacterial genes [15,16]. TM β-barrels are also formed by a number of bacterial pore-forming toxins in host membranes [17]. Single- and double-stranded β-helices were reported for membrane peptides with alternating L- and D-amino acids, such as gramicidin A, B, and C [18].

Due to progress in protein engineering, crystallization, and X-ray diffraction techniques, the number of integral membrane proteins with known three-dimensional (3D) structures is constantly growing [19]. It has currently reached more than 1750 entries in the Protein Data Bank (PDB) [20], or approximately 2% of the PDB content. Most of these entries (82%) correspond to TM α-helical proteins, less than 17% are TM β-barrels, and only around 1.5% are TM β-helices.

What can we learn from available protein structures about their membrane environment? What common features of TM α-bundles and β-barrels allow their general adaptation to the anisotropic lipid environment? What structural features can provide fine-tuning and specific adaptation of proteins to different types of membranes? What topological rules and membrane-sorting signals can be deduced from analysis of protein structures destined to different cellular membranes? Is it possible to characterize physico-chemical properties of different biological membranes with a complex protein and lipid composition based on the structures of their proteins?

To answer these questions, we examined 460 representative structures of integral and peripheral membrane proteins from our OPM (Orientations of Proteins in Membranes) database [21]. The current analysis significantly differs from previously performed studies of statistical distributions of residues in membrane proteins [22–28] in the following aspects: (i) we analyzed separately proteins from eight types of biological membranes using a sufficiently large dataset for each membrane type; (ii) proteins were positioned in membranes by the sufficiently accurate PPM method which has been extensively verified against numerous experimental data; (iii) we analyzed distributions of atoms rather than residues and only on the lipid-facing protein surface; and (iv) we implemented commonly used polarity descriptors of organic solvents (α, β and π*) to define polarity of protein surface and of lipid bilayers.

Analysis of protein atoms rather than whole residues improves the precision and statistical reliability of data: the greater number of atoms allows building the histograms with a 2 Å-step. Previous verification of the PPM method demonstrated a sufficiently high accuracy of calculated intrinsic hydrophobic thicknesses of TM proteins and their tilt angles relative to the membrane plane (1 Å and 2°, respectively), judging from deviations of these parameters in different crystal forms of the same protein [29]. Characterization of biomembranes by polarity parameters α, β, and π* has an important advantage because these parameters have a clear physical meaning as descriptors of dielectric properties and hydrogen-bonding. Besides, these parameters represent integral properties of different lipid-facing atoms and, therefore, are less sensitive than distributions of individual residues to structural and topological biases.

Based on calculated polarity profiles of membrane proteins and model lipid bilayers, we highlight the multilayered organization of the hydrocarbon core with a central nonpolar and two peripheral midpolar regions. We also identified polarity parameters and other structural properties that may reflect general and specific adaptations of proteins to eight different types of biological membranes. These results can be used to quantify anisotropic properties of the lipid environment in these membranes and to improve protein modeling methods.

2. Methodology

2.1. Overall approach to analysis of polarity of membrane components

The analysis of membrane proteins and lipid bilayers presented here is based on general approach to describe molecular solubility that was implemented in the upgraded PPM (Positioning of Protein in Membranes) method [29,30]. PPM allows calculation of binding energies and spatial positions of molecules of different sizes ranging from small organic compounds to large multi-protein complexes in membranes. The method was successfully validated using data for 24 TM and 42 peripheral proteins and many peptides whose arrangements in membranes have been experimentally studied [29–31].

The PPM method combines an all-atom protein structure with an anisotropic solvent representation of the lipid bilayer and the universal solvation model [32]. The solvation model describes the transfer energy of an arbitrary chemical compound from water to an organic solvent or another fluid environment. It accounts for long-range electrostatic interactions and first-solvation-shell effects (van der Waals, hydrophobic and hydrogen bonding interactions).

We found that the polarity of the solvent can be adequately described by a few commonly used parameters: its dielectric constant (ε), the solvatochromic dipolarity/polarizability parameter (π*) [33], and hydrogen-bonding donor (α) and acceptor (β) parameters of Abraham [34]. The α and β parameters have been previously used in SMx implicit solvation models developed for isotropic solvents [35]. We have extended this approach to anisotropic environments [30]. Accordingly, the lipid bilayer was represented as a fluid anisotropic solvent with polarity properties described by profiles of α, β, ε and/or π* parameters.

Hence, in the present work, we examined solubility properties of membrane proteins by calculating profiles of polarity parameters, α, β, and π*, for the lipid-facing surfaces of membrane proteins together with distributions of polar and nonpolar protein atoms, “hydrophobic dipoles” of Tyr and Trp residues, positively and negatively charged ionizable groups, crystallized lipids, detergents and water. The solvatochromic parameter π* replaces the macroscopic dielectric constant because it better describes microscopic dielectric properties of the environment and can be more easily calculated than the dielectric constant. In addition, we calculated polarity profiles for ten model lipid bilayers and compared them with profiles of membrane proteins.

2.2. Calculations of polarity profiles for model lipid bilayers

Transbilayer profiles of parameters α (z), β (z), and π*(z) and dielectric function F(ε)(z), describe the changes of polarity across the lipid bilayer [30]. These functions are used by the PPM method to define spatial positions of proteins in membranes. The profiles were previously calculated for the fluid dioleoyl-phosphatidylcholine bilayer (DOPC) using distributions of lipid quasi-molecular segments obtained from neutron and X-ray scattering data [36]. The concentration of water in the lipid acyl chain region of the DOPC bilayer was evaluated based on spin-labeling data [37].

Similar polarity profiles can be calculated for any other model lipid bilayer with known distributions of lipid components along the membrane normal. Here we compared ten lipid bilayers that have been previously studied in the fully hydrated fluid liquid-crystalline (L_α) phase that is biologically relevant (Table 2) [36,38–43]. Structural parameters for these bilayers were determined from X-ray scattering analysis, sometimes supplemented by a simultaneous fitting to neutron diffraction data [36]. The structure of each lipid bilayer is represented by Gaussian distributions of a number of lipid fragments with maxima indicating the most probable location of these fragments and width indicating range of their thermal motion along the bilayer normal. The distribution of water was obtained by subtracting concentrations of all