Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 5, Issue. 10, pp: 93-99, 2019
URL: https://arpgweb.com/journal/journal/16
DOI: https://doi.org/10.32861/jbr.510.93.99

Academic Research Publishing Group

Original Research **Open Access**

Characterization of Protein-Lipid Interactions in Biosystems Processes

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Abstract

Lipids correlate with membrane characteristics and functionalities as macromolecular constituentts in all cellular processes. Numerous aspects of lipid modulation of protein activity and structure are not completely understood and, thus a holistic systematic investigation activities will be pertinent. Protein-lipid interactions are the resultant impacts of membrane proteins on lipid physical states or vice versa. Encompassing research needs to be associated with strategies to elucidate whether proteins contain binding sites which are lipid specific, and that the protein-lipid complexes are ostensibly long-lived, on the time order necessary for the turnover of a normal enzyme. Biological membranes have since been determined as essential ingredients in an expansive array of cellular processes, such as photosynthesis, cell defence, signaling transduction, communication and motility. Therefore, they constitute multiple targets in both basic and applied research. Protein-lipid interactions are becoming increasingly relevant to the morphological characterization of membrane proteins as related to their functionalities. Excepting for simplified models, certain protein-lipid interactions specifically constitute remarkable challenges which require optimum experimental paradigm and design.

Keywords: Signaling; Transsduction; Membranes; Morphology; Biophysical techniques; Signaling; Challenges.

1. Introduction

The surge for understanding protein-lipid interactions has led to interests in the morpholigical characterization of the functions and structures of protein and lipids in biosystems processes. Lipids are in correlation with membrane characteristics and functions as molecular components in all cellular processes. Several aspects of lipid modulation of protein activity and structure are not completely understood, and thus, requires ardent holistic systematic investigation activities [1]. It has been demonstrated that proteins localized in rat brain myelin were soluble in organic solvents, such as chloroform methanol [2], and the term proteolipid was generated including the frequently applied technique for lipid extraction. It was revealed that these contained covalent-bonded fatty acids, and varied from plasma lipoproteins. Proteolipids are ubiquitous in nature, and are recognized as proteins having covalent-bonded lipid components which include isoprenoids, cholesterol, fatty acids and glycosylphosphatidylinositol; as well as non-covalently bonded lipid-protein complexes of the sort observed in plasma are referred to as lipoproteins. The terms lipoprotein, lipopeptide and proteolipid are applied interchangeably [3].

Protein-lipid interactions are the effects of membrane proteins on lipid physical states or vice versa. The relevant considerations to comprehending membrane structure and function include: (a) whether intrinsic membrane proteins are stringently bound to lipids and, what is the presenting nature of the lipid bilayers adjacent to the protein? (b) Whether membrane proteins possess long-range impacts on the dynamics or order of membrane lipids? (c) What are the mechanisms whereby lipids affect the protein membrane structure and/or functioning? (d) What are the mechanisms by which peripheral membrane proteins bound to the surface layer have interactions with lipids and affect their behavior? [4]. The heterogeneity of lipid constituents of biological membranes, and the impact of lipid macromolecules are currently being given due cognizance. Characterization on the morphology of membrane proteins depict that protein-lipid interactions employing investigative techniques is a daunting task that needs to be developed by meanss of newfangled strategy in order to elucidate the mechanism of membrane protein functionality.

2. Bonding Features

The full and proper functioning of cells are dependent upon biomolecular interactions within and between cells. The characterization of these molecular interactions has resulted in the elucidation of the molecular mechanisms of cellular control or regulation, biological processes, health and disease. Having complete grasp of the functionalities of every molecule of interest in the cell is invariably the objective of modern biology in nature, as they contribute to intermolecular interactions, both within a molecular class such as protein-protein-rna [5] or between classes as in protein-carbohydrate [6]. Whereas, the tools for these aforementioned classes are almost well established, the area of protein-lipid interactions remains relatively unexplored [7].

Most of the research has focused on cellular and subcellular structures (especially membranes), blood constituents, as well as with model system interactions in aqueous milieu, in tissues and selected food items [4]. Lipids interact with proteins via numerous types of bonding. In several select natural lipid-protein complexes, the lipoproteins have phospholipids in their lipid constituents. Phospholipid –protein interactions could be associated with electrostatic forces in which the binding can emanate when a negatively charged phosphate group and a positively charged group attract each other, such as lysyl or guanidyl residue. It could also include a positively

charged group in the phospholipid (as per choline) and a negatively charged residue (as per aspartyl). An identical binding mode is the production of salt bridges through divalent metals and calcium. It is possible that concurrent and cooperative stimulation of electrostatic binding and of salt bridges pertain in bacterial cell walls. The varied types of intermolecular forces involved are in decreasing order of magnitude: covalent > electrostatic > hydrogen bond > van der Waals. In broader terms, the bonding forces involved in protein-lipid interactions include: (a) van der Waal's which are non-polar bonds and act at short distances; (b) hydrogen bonds are polar bonds which are slightly strong forces derived from unsymmetrical sharing of electrons; and (c) charged entities which function as proton acceptors or donors and have the potential of attracting oppositely charged groups separated by appreciable distances [8]. Several membrane proteins selectively bind specific lipid species which have effect on precise insertions, folding as well as full and proper protein functionality [9]. Numerous peripheral membrane proteins are primarily bound to the membrane via interactions with the lipid bilayer surface. Certain of these, such as the myelin basic protein and spectrum mainly possess structural functions. A few water soluble proteins transiently bind to the lipid bilayer surface or within specific parameters. Misfolding processes, commonly exposing protein hydrophobic sites are usually linked with lipid membrane binding and concomitant aggregation, for instance, in neuroapoptosis, degenerative and neuronal perturbations [10].

3. Cellular and Dynamic Processes

Biological membranes have since been determined as essential ingredients in an expansive array of cellular processes, such as photosynthesis, cell defence, signaling transduction, communication and motility. Therefore, they constitute multiple targets in both basic and applied research [11], in PC and PG. The QA/QA- redox potential charges determined by delayed luminescence revealed (a) a differential lipid effect of RC entrenched in micelles or vesicles; (b) ma binding interaction alteration between anionic lipids and RC; (c) a particular effect of PC and PG on the free energy levels of the primary and secondary quinines ostensibly via the network of intraprotein hydrogen bonding; and (d) substantial augmentation of the QA/QA- free energy in PG than in PC both in single-component vesicles and detergent micelles. These provide research basis for structural data, implications of the binding attributes to RC and likely interactions between lipids and electron transfer components [12, 13]. The interactions between peptides and lipids are essentially important in the action of a plethora of membrane-mediated cellular processes, such as antimicrobial peptide function, hormone-receptor interactions, ingress via the blood brain barrier of drug bioavailability, and viral fusion. Furthermore, a principal objective of current biotechnology is access to novel potent pharmaceutical agents having biological action that depends on peptide binding to lipid layers. Research must focus on secondary orientation, oligomerization and structure localization within the membrane. Simultaneously, it is necessary to elucidate the structural impacts from the peptides on the lipid bilayer because they are significant for the interactions. The structural active peptide membrane characterization is an arduous investigational task. It is perspicuous that a single experimental method or design can provide an encompassing structural characterization of the interaction excepting a multidimensional trajectory is undertaken. Diverse peptides empl0y varied interaction mechanisms or combinations of mechanisms; and the interaction mechanisms can change with respect to pH, temperature and peptide content of the system. To understand the biological interaction processes between peptides and membrane or peptide design with determined functionalities and specificities, it is pertinent to configure with molecular resolution the association between the morphological characteristics, such as hydrophobicity dissemination or the peptide charge as well as the specific interaction mechanism induced. The importance of peptide-membrane interactions at the molecular level is relevant to elucidate and explicate diverse biological processes in order to design peptides with specific functionalities as in antibiotic and drug delivery regimens. [14-17].

Bacterial lipoproteins characteristically present conserved N-terminal lipid-modified cysteine residue providing the hydrophilic protection linkage access to bacterial cell membranes. These proteins are relevant in expansive bacterial physiological processes, such as virulence; and induce innate immune reactions, as ligands of the mammalian Toll-like receptor 2. Recent work has revealed three lapidated lipoprotein structures in monoderm bacteria: the lyso, N-acetyl and peptidyl configurations. The bacterial lipoprotein structure is normally constant in every bacterium, but in Staphylococcus aureus, they differ between the diacyl and triacyl varieties depending on the ambient conditions. Therefore, the lipidation conformation of bacterial lipoproteins, especially in monoderm bacteria exhibit greater cpmplexity than was presumed. Phospholipase Ds (PLDs) and PLD-derived phosphatidic acids (Pas) have relevant functions in plant hormonal and environmental elicitations and diverse cellular dynamics. The molecular varieties and redundant functionalities cause PLD-PA to be a relevant signaling complex that regulates lipid metabolism, cytoskeleton dynamics, hormonal signaling and vesicle trafficking during plant defence via protein-protein and protein-lipid interactions or hormonal signaling [18].

4. Osmosensing and Osmoregulation

Osmosensing and osmoregulation constitute critical life mechanisms for all living cells. The bacterial cell wall cytoplasm normally comprises 300-400g/l of macromolecules, typically proteins, DNA and RNA which occupy portion of the cellular volume [19]. Bacteria do function in the maintenance of their hydration during environmental alterations as pertain to their osmotic pressure. As the external osmotic downshift (osmolality reduction) occurs, mechanosensitive channels become activated and release low relative molecular mass osmolytes combined with aqueous medium from the cytoplasm. With osmotic upshift, osmoregulatory transporters are activated to allow ingress of molecules with water. Osmoregulatory channels and transporters detect and elicit reactions to osmotic

stress through diverse mechanisms. Mechanosensitive channel, Mscl senses the accelerating tension in the membrane and appears to gate as the lateral pressure within the acyl chain region of the lipids decreases to less than a threshold level. Transporters OpuA, BetP and ProP become activated as elevated external osmolality results in threshold ionic levels greater than circa 0.05M when measured at the proteoliposome lumen. The threshold activation levels for the OpuA transporter significantly rely on the anionic lipid fraction that encompasses the cytoplasmic protein presentation. The more elevated the anionic lipid fraction, the more increased the threshold ionic contents. An identical trajectory is detectable for the BetP transporter. The lipid reliance for the osmotic activation of OpuA and BetP translates those osmotic signals may be transmitted to the protein by interactive mechanisms associated with charged osmosensor domains and the ionic headgroups of the membrane lipids. The charged C-terminal BetP and ProP domains are relevant for osmosensing. The C-terminal domain of ProP is associated with homodimeric coiled-coil production and interaction with the membrane lipids as well as soluble protein ProQ. The ProP activation by luminal, macromolecular solutes at consistent ionic strength suggests that its structure and activity also respond to macromolecular crowding. The excluded volume impact could cause restriction of the range in excess of which the osmosensing domain is likely to have electrostatic interaction. An adequately simplified view posits a theory of dissociative double layer that explicates transporter activation as to how alterations in ion contents modulate interactions between charged osmosensor domains and charged protein or lipid surfaces. Significantly, the relatively elevated ionic levels wherein osomosensors are activated at various surface charge densities correspond with the predicted dependence of critical ion levels on surface charge density. The critical ion levels are representative of transactions in Maxwellian ionic presentations where the surface potential measures 25.7mV for monovalent ions. The osmosensing mechanism is qualitatively depicted as an “ON/OFF switch” relating to thermally relaxed and electrostatically bound conformations of protein [20-26].

5. Antimicrobial Attributes

Antimicrobial peptides are naturally strategized longstanding host-defence components in all life domains. They constitute part of the innate immune system and fight bacteria, fungi, viruses and carcinogenic cells. Peptides present structural and mechanistic variations, but several of them directly act at the cell surface with predilection on lipids or membrane bound components, such as cell wall precursors. Most mechanistic research regarding the antimicrobial mechanisms of membrane-directed peptides have been invariably related to their interaction with artificial lipid systems [27]. Diverse models of mode of action have been posited, however, classical pore producing models, such as the barrel-stave, toroidal pore or carpet models make provision for interaction of amphipathic alpha-helical peptides with lipid bilayers. These models are not appropriate for smaller or more compact peptides which are incapable of spanning the lipid bilayer and produce a pore. Alternative interpretations for pore production are: (a) molecular ectroporation, (b) sinking raft, (c) interfacial action, and (d) lipocentric pore generation models, with application to all smaller AMPs [28, 29]. Inasmuch as theses studies are amenable for understanding AMP interaction with lipid bilayers, the translation of these models into a natural in vivo presentation may be cumbersome. Biological membranes present more lipid complexity than can be configured similarly in model research. Also, biological systems are extremely dynamic and their membranes differ considerably as regards ambient conditions. Lipids of declined or elevated fluidity and membrane curvature are relevant in membrane action. Significantly, a biological membrane is not available merely as lipid components but as much as 60 per cent protein; a situation that is usually neglected when explicating AMP membrane functionality. There is a paucity of in vitro studies concerning AMP function, and are restricted to characterizing their pore-generating attributes. Pore production is concentration dependent, and usually sub pore-producing doses are constitutively inhibitory. There are minute AMPs which do not influence membrane permeability. Therefore, the inhibitory interactions occurring at the membrane interface are not amenable to full and proper interpretations with the presenting pore production models [30]. To grasp the in vivo mechanism of AMP action, it is pertinent to visualize biological membranes holistically, taking into consideration the membrane arrangement and protein niche. Thus, knowledge and information unification of in vivo and in vitro research of model systems may create a sustainable paradigm on the physiological resultant impact on the targeted cells on both lipid and protein components of biological membranes. This functionality can be correlated with diverse charge dissemination on peptide amino acid sequences [31, 32].

RWRWRW-NH2 (MP196) is an amphipathic hexapeptide thattargets bacterial cytoplasmic membrane with resultant inhibition or restriction of cellular respiration and cell wall synthesis, with potent action against Gram positive bacteria without perspicuous cytotoxicity or haemolysis or haemolysis as revealed in erstwhile studies. Thus, MP196 is employed as primary structure to develop increasingly potent antibiotic derivatives. It is suggested that MP196 derived directly further decreases haemolysis and may influence to ameliorate acute toxicity. Haemolysis assessment is critical in clinical evaluation of future applicable antimicrobial peptides undergirded with microscopy-based blood cell morphological analysis [33].

The molecular functionality of polyene macrolides with antifugal action, amphotericin B and natamycin integrates sterol membrane recognition. Physicochemical and functionality investigations have assisted immensely to elucidate and explicate the interactions between amphotericin B and ergosterol and, to a perceptible degree also, with cholesterol. Conversely, there is extant paucity of molecular details on interactions between natamycin and sterols. At the molecular level, high resolution solid state 13CMAS NMR from natural abundance 13C is an important instrument to investigate drug-target interactions within lipid membranes [34]. Evidence of alterations in sterol core and chain motion suggests certain magnitude of similarity between the molecular complex generated between amphotericin B or natamycin and any of the sterols. Although, amphotericin ostensibly engages cholesterol transiently flat on the ring structure without perturbing ring methyls, and to engage the chain terminals. In

contrast,its interaction with ergosterol exposes alterations in ring methyl 19 and depicts chain double bond engagement. Natamycin has interactions with both sterols engaging stringently the ring A of cholesterol, but transiently with other sterol ring, and all ring methyls which are directed to one aspect of the sterol core including chain base methyl 21 and the cholesterol chain terminal. Complex formation with ergosterol demonstrates a common engagement of sterol ring with numerous contacts, encompassing sterol chain engagement but not ring methyl.

The most remarkable contrast between amphotericin B and natamycin action is that the larger amphotericin B molecule cooperatively engages lipid/sterol mixed membranes, thus influencing the membrane arrangement and stabilization; whereas natamycin presents stringent and specifics interactions with both sterols. The contrastingly decreased cooperativity of molecular interactions in natamycin–containing membranes provides the latitude for non-perturbation of membrane morphological characteristics. With respect to the minimal therapeutic natamycin requirement or level in contrast to membrane cholesterol, specific interactions have slight impact on the cholesterol fraction present. Conversely, reduced ergosterol molarity is critically affected by natamycin, resulting in the deregulation of ergosterol-dependent protein action [34].

It has been determined that membrane thinning is a fundamental mechanism via which antimicrobial peptides can derange cellular membranes. Results suggest that diverse factors influence the resultant alterations in membrane thickness, such as bilayer peptide orientation as well as incontrovertible hydrophobic mismatch to bilayer adaptation [35].

6. Biophysical and other Analytical Techniques

Robust research is associated with strategies to elucidate whether proteins contain binding sites which are lipid specific, and that the protein-lipid complexes are ostensibly long-lived, on the time order necessary for the turnover of a normal enzyme, that is usually 10-3sec. This is realizable via the application of 2H-NMR, ESR and fluorescent methodologies. Two strategies are applicable to determine the relative affinity of lipids to be bound to particular membrane proteins. These involve the application of lipid analogues in reconstituted phospholipid vesicles having the particular protein. The mobility of spin-labeled phospholipids is restricted when adjacent to membrane proteins, resulting in a broadened component in the ESR spectrum. Analysis of the experimental spectrum as the totality of the two components depicts an accelerated tumbling species in the aggregated lipid phase with a defined spectrum, and a mobility restricted component adjacent to the specific protein. Membrane protein denaturation results in extrapolated widening of ESR spin-label spectrum with further undergirding regarding protein-lipid interactions [36]. Spin-labeled and brominated lipid derivatives can quench the intrinsic tryptophan fluorescence from membrane proteins. The quenching efficiency is dependent on the distance between the lipid derivative and the fluorescent tryptophans. Several 2H-NMR studies with deuterated phospholipids show that protein presence is of minimal consequence on either the order parameter of the liquid within the bilayer or the dynamics of the lipids as determined by relaxation times. The encompassing perspective from NMR experiments include: (a) there exists accelerated exchange rate of 107sec-1 between the boundary and free lipids; (b) the order parameter of lipids bound are rarely influenced because they are adjacent to proteins; (c) there is an extant negligible retardation in the frequency range, 109sec-1 of the dynamics of the acyl chain reorientations; and (d) the orientation and dynamics of the polar headgroups are not affected to the same substantial magnitude by being adjacent to transmembrane proteins. In addition, 13C-NMR spectrum provides information on specific membrane protein-lipid interactions [37]. Results of non-labeled optical methods, such as Dual Polarisation Interferometry measuring the birefringence [38] and/or order within bilayers have demonstrated peptide and protein interactions impact on bilayer order, with particular emphasis on the real time association to bilayer and cortical peptide level, culminating in the penetration and disruption of the bilayer order by peptides [39]

Thorough experimental and computational approaches to detect and analyze protein-lipid interactions are needed. There are broadly two main experimental technology categories, such as solution-based and array-based methods; while computational methods involve large-scale data, robust analyses, and predictions/dynamic simulations derived from innate knowledge and information of experimentally determined interactions [7]. Progressive development in these experimental technologies has resulted in sustainably enhanced computational analyses and reciprocally, thus promoting the understanding of protein-lipid interactions and their relevance in biosystems.

Eukaryotic cells contain numerous diverse membrane compartments with characteristic dynamics, lipid organization and shapes. A vast majority of the fractions of cytoplasmic proteins are involved with these membrane compartments. These protein-lipid interactions control or regulate the subcellular niches and actions of peripheral membrane proteins to the extent, from cytoskeleton dynamics via membrane trafficking to intracellular signaling [40]. In reciprocity, several membrane-associated proteins modulate the shape, lipid composition and dynamics of cellular membranes. Elucidating the specific mechanisms of protein-membrane interactions will be of immense benefit to explicate their biological functions [30]. A series of fluorometric centrifugation and microscopic assays are currently available to study the molecular mechanisms of protein-membrane interactions, and the impact of these interactions on the conformation and dynamics equally on protein and lipid bilayers. These arrays specifically provide disparate sorts of data with their peculiar limitations. It is imperative to select an assay that corresponds with the structural and chemical attributes of the specific protein to provide the sort of information required. Since lipid organizations differ markedly between specific cell types, their membrane compartments and both leaflets of each membrane, it is pertinent to select the lipid composition of the model membrane so that it has a resemblance to the specific native membrane compartment as to provide reliable, significant knowledge and information in protein-lipid interactions [18].

7. Discussion

Protein-lipid interactions are becoming increasingly relevant to the morphological characterization of membrane proteins as related to their functionalities. Excepting for simplified models, certain protein-lipid interactions are specifically challenging to experimentally highlight. A study employed molecular dynamics simulation for the identification of a specific protein-lipid interaction in lactose pernease, a prototypical transmembrane protein model [41]. The interactions are able to be correlated with action reliance of the protein to lipid specificity. The tool is not complex, and it is expansively amenable to other membrane proteins; and diverse lipid matrices are utilizable [41]. The integrated relationship between proteins and lipids triggers several significant cellular processes, such as membrane trafficking, signal transduction and cytoskeleton remodeling. Membrane trafficking applies the Golgi complex as its hub, and drives the biosynthesis, transport and intracellular dissemination of numerous proteins and lipids. Its structure and function are directed via intimate functional relatedness between protein-based and lipid-based organizations. These machineries are associated with the control of the essential events which effect membrane traffic, such as observed in budding, fusion and fission intermediates in the control mechanisms of the geometric morphology of the specific Golgi membranes, and ultimately, in signal elicitations which are localized in the secretory systems, or that may influence other cellular systems. Lipid-protein interactions depend on the potential specific domain lipid recognition. These domains are particularly mediated via the phospholipid headgroups; however, certain of these protein domains can specifically interact with the phospholipid acyl chains. It is evident that specific proteins and/or protein domains have greater sensitivity to the physical ambient of the membrane layer, such as curvature than its chemical arrangement [42]. Membrane lipids are significant in the morphology of membrane-embedded transporters [43].

It is suggested that the cell membrane is a patchwork structure comprising several proteins and lipids which do not entirely diffuse but are restricted to dynamic microdomains within the membrane plane. These domains can form or be sustained in diverse manners, for instance, "lipid shells" encumbering proteins and/or cytoskeletal compartmentalization [44]. In addition to micro-environment interactions which co-localize multiple moieties of certain functional units, these may be connected to the activity of the unit [44]. Functionally active membrane peptides specifically target membranes instead of receptor proteins and conduct their bioplogical roles by cooperative functionality [45] Biophysical characterization is necessary to elucidate the entire mechanism of peptide-membrane interactions.

8. Conclusion

Protein-lipid interactions are governed by the first principles of universal biochemical and physicochemical ruless which are applicable to every tissue. These protein-lipid interactions are linked with the preservation or sustenance of the functionality of integral biosystem proteins. A variety of protein-lipid interactions may prevail at the membrane surface , whereby peripheral membrane proteins alter the membrane curvature, or stabilize a presenting specific membrane curvature. Further studies are necessary for the determination of essential biochemical and biosynthetic pathways in protein-lipid interactions as related to cellular processes, such as photosynthesis, cell defence, signaling transduction, communication and motility as they are associated in individual and multiple targets in both basic and applied research.

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