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Role of guanidinium group in the insertion of L-arginine in DMPE and DMPC lipid interphases

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ABSTRACT

L-Arginine (Arg) is a positively charged amino acid constituent of peptides and proteins, participating in diverse mechanisms of protein–membrane interaction. The effect of Arg on phosphatidylcholine (PC) membranes has been previously related to water structure changes and to the presence of water defects in the hydrocarbon region. However, no information is available with regard to phosphatidylethanolamine (PE), another important component of lipid membranes. For this reason, the aim of this study is to determine the effect of Arg on DMPE membranes and partially methylated PEs in comparison to DMPC. The adsorption of the amino acid onto the lipid membranes was followed by determining the changes in the surface potential as a function of the bulk amino acid concentrations. The effects of Arg on the surface properties were also measured by changes in the surface pressure and the dipole potential. The onset of the transition temperature was measured with a fluorophore anchored at the membrane interphase. The results provide a new insight on amino acid—PE interactions, which can be ascribed to specific perturbations in the head group region induced by the guanidinium residue.

1. Introduction

The interaction of proteins with lipid membranes remains of central interest in biophysical research. Understanding a wide range of fundamental molecular mechanisms, such as the action of antibiotic peptides, the association of proteins involved in cell signaling and membrane fusion and also nongenomic action of some hormones is based on a molecular interpretation of the interaction of constitutive amino acids with the lipid matrix [1].

Several studies have considered the effect of the lipid composition on the adsorption, penetration and intercalation of functional and structural proteins and its consequences on the enzymatic activity and membrane structure [2–4]. Several homo- and hetero-synthetic peptides have been used as model systems to elucidate the effect of either naturally occurring or synthetic pore-forming peptides. A great emphasis was put on correlating the penetration of the different peptides into the membrane with the phase properties and the domain formation of lipid mixtures [5]. In general, the interaction of proteins with different types of membranes has been explained in terms of the insertion of some amino acids at different depths of the bilayer affecting the hydrocarbon core [6,7]. In this regard, some models postulate the partition of individual amino acids composing the protein of interest into different regions of the bilayer. Thus, thermodynamics of lipid–peptide side chain interactions becomes a critical step for clarifying the stabilization of amino acid side chains into lipid bilayers [2].

It has been suggested that flanking residues of transmembrane segments might influence the positioning of membrane proteins at the membrane interface [8]. This implies the presence of specific sites near the surface for defined amino acids. For instance, it was shown that the binding of polycationic peptides is mainly due to electrostatic interactions and that small peptides do not bind to membranes formed from electrically neutral lipids like PC [9]. However, this binding seems to be due not only to electrostatics [10]. In this regard, other studies suggest the participation of specific interactions of polycationic peptides with phosphocholine head groups [11]. The TAT (peptide rich in arginine) induces the formation of rodlike, presumably inverted micelles in DMPC, which may represent intermediates during the translocation. The molecular interactions responsible of this mechanism seem to involve the formation of complexes between the phosphate group and the arginine side chain [11]. Therefore, in order to understand the mechanism of peptides insertion, it appears of interest to have an insight on the effect of isolated amino acids on the lipid surface and phase properties of membranes of different lipid composition.

Among positively charged amino acids, L-arginine (Arg) is an important component of several peptides and proteins. This amino acid exposes a guanidinium group at the end of an apolar region in addition to the amine and carboxilic groups. There has been great interest in recent years concerning the protonation state of Arg residues in a lipid bilayer environment. This interest was partly triggered by the observation that several Arg residues on the S4 helix may come in contact with the hydrophobic region of the lipid

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membrane in a crystal structure of the potassium channel [12]. In addition, the interaction of the isolated amino acid has also received some attention. It was reported that isolated Arg is transported and accumulated in different types of cells, strongly suggesting the ability of this molecule to be transported through cells or plasma membrane vesicles [13–15]. In connection with these properties and functions, it should be mentioned that enhanced transport of L-arginine in smooth muscle cells [16] occurs when L-arginine is encapsulated in liposomes. This amino acid is associated with the generation of nitric oxide (NO) in living organisms and is involved in endothelial dysfunction associated with atherosclerosis, diabetes and other diseases [17,18].

Molecular dynamics studies, undertaken to achieve an understanding of the mechanism of partition, have shown that Arg may be either charged or uncharged at the center of PC membranes [19]. It has been suggested that this may be due to the formation of water defects connecting the side chains to bulk water [7]. Thus, the energetics of partitioning assumes that the process of Arg interaction with PCs involves the presence of water in the membrane structure. In consequence, the thermodynamics of this process is complex since burying a charge in the membrane involves protonation/deprotonation in bulk water and in the membrane. In turn, deprotonation of Arg may involve lipid membrane deformation and changes in the water structure due to the Arg charge electrostriction [19]. The polar headgroups of the lipids can stabilize the charged Arg residues in the membrane, causing the lipid membrane to deform and dehydrate locally [3]. This implies that the hydration properties of the lipid components of the membrane may regulate the amino acid partitioning. Therefore, the possible effects of Arg could be related to the hydration level of the membrane lipid components. If this is the case, effects of the amino acid could be different in membranes composed of lipids having different affinity for water, such as phosphatidylcholines and phosphatidyl ethanolamines.

The hydration rates of these lipid components are related to the fluctuations at the water–hydrocarbon interphase of the carbonyl groups and the exposure of the phosphate groups to the aqueous media [20]. This affects the compressibility and area per molecule, which appears important in the mechanism of Arg partition, as discussed above. In this particular, PEs may adopt different surface area and special arrangements, due to its molecular shape and to the strong lateral head group interactions due to the formation of H bonds [41].

In this regard, amino acids can be considered H-bonding compounds that may interact with membrane surface groups similarly to sugars and polyphenols, replacing water in the hydration sites [21–26]. Thus, in order to gain insight into the molecular interactions of Arg with membranes with different states of hydration, the effects on surface and dipole potentials have been investigated by means of surface pressure curves and fluorescence methods in DMPC and DMPE interfaces. In particular, the role of the hydration centers, carbonyl and phosphate groups in the two lipids, is of special interest since they are involved in the determination of the surface potentials, such as charge and dipole potential [27]. Constitutive groups of a lipid interface such as P=O and C=O groups and the water molecules polarized by them determine the dipole potential of lipid membranes [21,28]. For these reasons, we have investigated the effect of Arg on the zeta potential, dipole potential and surface pressure of monolayers and bilayers of different derivatives of saturated phosphatidylcholine (DMPC) and phosphatidyl ethanolamine (DMPE). Thus, changes in the zeta and dipole potential may be related to the binding of the amino acid to these groups.

Furthermore, the conformational changes and water content at the polar head groups may modulate local changes in the dielectric constant of the bilayer [29]. For this purpose, structural parameters at the lipid interphase and the hydrocarbon region were measured by fluorescence methodologies for different concentrations of Arg in PC and PE membranes.

2. Materials and methods

2.1. Chemicals

1,2-Dimyristoyl-sn-glycero- 3-phosphocholine (DMPC), 1, 2-di-*O*-tetradecyl-sn-glycero-3- phosphocholine (etherPC), 1,2- dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE), 1,2-di-*O*-tetradecyl-sn-glycero-3-phosphoethanolamine (etherPE), 1,2-dipalmitoyl-sn-glycero-3-phospho-ethanolamine *N*-monomethylated (mmDPPE) and *N*,*N*-dimethylated (dmDPPE) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and used as received. The purity of lipids was checked by thin layer chromatography using a chloroform:methanol: water mixture as running solvent.

L-Arginine (Arg) was obtained from Sigma-Aldrich (Saint Louis, MO).

The fluorescence probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and 6-dodecanoyl-2-dimetilaminonaftaleno (Laurdan) were obtained from Molecular Probes and used as received. Chloroform and KCl were analytical grade. Water was MilliQ quality.

The pHs of the solutions for zeta and dipole potentials were adjusted by titrating with HCl or with buffer carbonate when necessary.

2.2. Monolayer formation

Dipole potential and surface pressures were determined in lipid monolayers. Aliquots of chloroform solutions of the different lipids were spread on a clean surface of water, or on aqueous solutions containing Arg at the different concentrations tested. Data were collected when constant potential or pressures were reached and no changes were observed with further additions of lipids. In this saturation condition, the lipids in the monolayer are in equilibrium with lipids forming liposomes in the subphase. In this condition, a corresponding state between bilayer and monolayer is achieved since equilibrium is established by the transfer of lipid molecules to and from the monolayer and the outer monolayer of the vesicles [30,31]. Both experiments were performed at the same temperatures and conditions.

2.2.1. Determination of dipole potential in monolayers

The values of interfacial potential (V_{surf}) were determined through a high impedance circuit, by means of an ionizing electrode on the monolayer and a reference electrode in the aqueous subphase (KCl 1 mM) using the following expression:

$$V_{surf} = V_{Ag/AgCl} - V_{grd} = V_{solution} - V_{grd},$$

where $V_{Ag/AgCl}$ is the potential of the reference electrode and V_{grd} is the potential of the shield covering the ionizing electrode.

Temperature was set at the values indicated in each assay (mostly 18 and 28 °C) and measured with a calibrated thermocouple immersed in the subphase and maintained within ±0.5 °C.

The dipole potential of the monolayer (Ψ_D) was evaluated as

$$\Psi_D = V_{surf} - V_{lip} \quad (1)$$

where V_{surf} is the potential of the clean surface (without lipids) and V_{lip} is the potential after the monolayer was formed.

Different values of Ψ_D were obtained for the clean surface of the amino acid solution assayed and with a monolayer of lipids, in the conditions described below. These values are reported as a function of the amino acid concentration in the subphase solution [32].

2.3. Surface pressure measurements in monolayers

2.3.1. Area per lipid calculation

The formation of saturated monolayers of lipids, on the interface of solutions with and without amino acid, was monitored by measurements of the surface pressure of the different lipid monolayers in a