THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

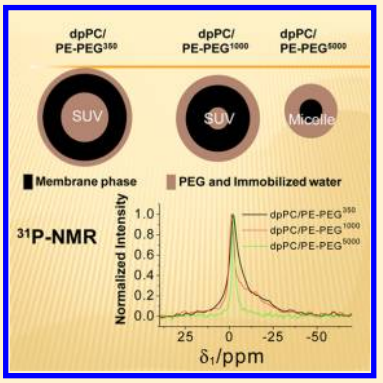
Water and Membrane Dynamics in Suspensions of Lipid Vesicles Functionalized with Poly(ethylene glycol)s

Eduardo M. Clop,[†] Ana K. Chattah,[‡] and María A. Perillo*,[†]

[†]IIByT (CONICET-UNC), Cátedra de Química Biológica, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina

[‡]Facultad de Matemática Astronomía y Física (FaMAF) and IFEG (CONICET-UNC), Universidad Nacional de Córdoba, Ciudad Universitaria, X5016LAE Córdoba, Argentina

ABSTRACT: The present work was aimed at studying the molecular dynamics at different levels of model membranes having a simulated glycoclix, with focus on the molecular crowding conditions at the lipid−water interfacial region. Thus, binary mixtures of dipalmitoylphosphatidylcholine (dpPC) and a poly(ethylene glycol) (PEG^n) derivative of dipalmitoylphosphatidylethanolamine (PE) (where n = 350, 1000, and 5000, respectively, refer to PEG molecular masses) were submitted to ^{1}H spin−lattice relaxation time (T_1) and ^{31}P NMR spectra analysis. ^{1}H NMR relaxation times revealed two contributing components in each proton system (PEG, phospholipids, and water), for all the mixtures studied, exhibiting values of T_1 with very different orders of magnitude. This allowed identifying confined and bulk water populations as well as PEG moieties becoming more disordered and independent from the phospholipid moiety as n increased. ^{31}P spectra showed a typical broad bilayer signal for n = 350 and 1000, and an isotropic signal characteristic of micelles for n = 5000. Surface pressure (π)−molecular area isotherms and compressional modulus measurements provided further structural information. Moreover, epifluorescence microscopy data from monolayers at $\pi \sim 30$ mN/m, the expected equilibrium π in lipid bilayers, allowed us to postulate that both ^{1}H populations resolved through NMR in phospholipids and lipopolymers corresponded to different phase domains.



INTRODUCTION

Biochemical processes occur at a high global concentration of macromolecules, between 50 and 400 mg/mL (5−40% W/V).[1−3] This is a distinguishing feature of living systems usually described as molecular crowded (MC) environments. Within MC media, macromolecules occupy a large fraction of volume that cannot be occupied by other molecules.[4] Thus, large molecules are confined due to their big size compared with the pore size[5,6] and the volume accessible to small molecules is significantly lower than under conditions of dilute solutions, where most experiments are done. As a result, molecules are subjected to steric and diffusional restrictions affecting their properties.[7] Moreover, in MC systems, a significant population of water molecules remain tightly bound to the crowding species, thus being sequestered from bulk solvent.[8]

The cellular glycocalix (GC) is a typical example of a MC environment. The GC is a polysaccharide protective layer that conforms the environment where membrane anchored proteins are embedded.[9,10] It contributes to the antiadhesive properties of cells under normal disease free conditions and may also control the accessibility of solutes (e.g., drugs) to the cell interior and hinder the binding of targeted carriers to the cell surface.[11] The compactness of the GC is expected to affect its structure and hydration conditions. Consequently, studies on water dynamics within the molecular crowded environment of a GC would be of upmost importance to understand physical and biochemical phenomena taking place at the membrane interfacial region.

In this regard, nuclear magnetic resonance (NMR) through spin−lattice and spin−spin relaxation times has proven to be a very useful technique to provide information on aqueous solutions, on a variety of time scales and solute concentrations.[12−15] In addition, it is a noninvasive method that can be applied to a wide spectrum of solution samples. In particular, it has allowed identification of the dynamics of different water populations, helping to distinguish free liquid fractions from structured water in the presence of solutes.[16] Additionally, 1D ^{31}P NMR spectra have been extensively used to study the coexistence of different types of supramolecular aggregates (e.g., micelles and vesicles).[17,18]

Using poly(ethylene glycol) (PEG) in solution, it is possible to achieve MC conditions.[4,19,20] The ability of PEG to fulfill this role has been attributed mainly to its physical properties, such as solubility in water at all concentrations, a large exclusion volume, and a high degree of conformational entropy.[21−23] Recently, from NMR measurements of spin−lattice relaxation times of PEG^{6000} solutions, we reported the presence of two molecular populations, both in water and in PEG, as well as a PEG^{6000} aggregation also supported by dynamic light scattering

Received: November 5, 2013
Revised: May 6, 2014
Published: May 8, 2014

   dx.doi.org/10.1021/jp410894x | J. Phys. Chem. B 2014, 118, 6150−6158