

Lipid monolayers on Hg as a valid experimental model for lipid membranes under electrical fields

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Received 7 September 2005; received in revised form 9 December 2005; accepted 12 December 2005

Available online 29 December 2005

Abstract

Monolayers spread on Hg drops are shown as a suitable experimental set up to study the influence of external electric fields on the structure of lipid membranes. The electrical response exhibits a sharp transition at 24 °C, the transition temperature of DMPC. In addition, voltammetric response of monolayers of mixtures of DMPC/DMPE adsorbed on mercury, shows a similar trend to that found for dipole potential of monolayers of the same composition spread on an air–solution interface.

It is concluded that a lipid monolayer adsorbed in a mercury–solution interface, has comparable properties as those found in other experimental models of lipid membranes in similar conditions. In addition, they constitute an ideal set up to study the effect of electrical fields on the dynamic conformation of lipids as a function of packing change produced by the condensation in the gel state or by the interaction of polar head groups.

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Keywords: Lipid membranes; Electrical fields; Lipid monolayers; Hanging drop mercury electrode; Cyclic voltammetry

1. Introduction

Lipids spread on the air–water interface stabilized as monolayers have been used extensively as experimental model systems to investigate the properties of biological membranes. It is well known that the phase transition between condensed and liquid expanded states can be achieved at fixed surface pressures in which the cohesion

between the lipid molecules gives place to a cooperative phenomenon involving all the monolayer molecules. The condensed state in the monolayers corresponds to the gel state in bilayers and the expanded state to the liquid crystalline in the bilayers of the same chain length (Lundkvist, 1978; Phillips et al., 1969; Mouritsen, 1991).

Mechanical and thermodynamic properties of bilayers, particularly phase-transition parameters, correspond closely to those of monolayers with which they are in equilibrium. However, questioning have raised when monolayers are compared both with planar bilayers (BLMs) and those from dispersed systems such as liposomes or vesicles. In the first case, the dipole potential measured in vesicles or plane bilayers using probe techniques, are approximately 100–150 mV lower

Abbreviations: DMPC, dimirystoylphosphatidylcholine; DMPE, dimirystoylphosphatidylethanolamine; HDME, hanging drop mercury electrode

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than dipole potential measured in monolayers of the same composition by a ionizing electrode (Franklin and Cafiso, 1993; Smaby and Brockman, 1990).

On the other hand, it has been argued that bilayers in vesicles are intrinsically comparable to monolayers since they show the phase transition at similar temperatures (Simon et al., 1975; Mac Donald and Simon, 1987; Blume, 1979). A sharp decrease in the dipole potential of the monolayer of dimirystoylphosphatidylcholine (DMPC) was observed at 24 °C, the same at which a decrease occurs in the turbidity of a sample of the same lipid dispersed in water.

Although reasonable, it may be questioned that this conclusion is drawn from the comparison of a surface property in a monolayer (the dipole potential) and a bulk property (the index of refraction) in bilayers of a multidispersed system. Based on these questionings, the surface properties in monolayers and bilayers were compared in mixtures of different types of lipids. It has been shown that the dipole potential of mixed lipid monolayers gives a complex but reproducible pattern as a function of the molar fraction of lipids of different chain lengths. A comparable pattern was observed for the same lipid ratios for the zeta potential (surface potential) of the external face of bilayers of liposomes (Luzardo et al., 1998).

Lipid monolayers can also be formed on the surface of mercury drops and used as experimental set up to study the influence of external electrical fields on the structure of lipid membranes. For this reason, it seems appropriate to validate this experimental model in order to determine whether their phase and surface properties are comparable to those found in other well established lipid model systems mimicking biological membranes.

Previous studies (Miller et al., 1992; Lecompte and Miller, 1980; Nelson and Benton, 1986) showed that monolayers on Hg provide an experimental membrane model which has high mechanical stability and resistance to high electric fields in comparison to BLMs.

The dependence of the surface dipole potential with the charge density of self-assembled monolayers of phosphatidylserine and phosphatidic acid on Hg, has been determined by means of differential capacitance and charge density measurements (Moncelli et al., 1998). The sigmoidal curve obtained shows an inflection point at zero charge density which, in opinion of the authors, is strongly reminiscent of the surface dipole potential that results from the reorientation of adsorbed water molecules at electrified interfaces. They have also observed a small increment of capacitance when the frequency is below 80 Hz. They explain this result as due to a sluggish collective reorientation of some polar

groups of the lipid. Besides, it has been determined the dependence of the charge density of self-assembled monolayers of different lipids on Hg, as a function of pH (Moncelli et al., 1994; Moncelli and Becucci, 1995).

Monolayers on Hg drops have also been extremely useful to study the response of lipid membranes to electrical potentials considering them as an environment in which proteins can insert (Miller et al., 1992; Lecompte and Miller, 1980; Nelson and Benton, 1986). In these cases, the properties of the species hold in the monolayer, such as, the charge transference across protein channels, the behavior of redox couples, and the formation of pores has been studied by means of cyclic voltammetry (Becucci et al., 2002; Bizzotto and Nelson, 1998; Gordillo and Schiffrin, 2000).

However, it has not been clearly shown that monolayers on mercury surfaces bear similar physicochemical properties, such as: phase state and membrane dipole potential of mixtures of different phospholipids, as other experimental model systems of lipid membranes. In addition, it is also of interest to investigate if studies of cyclic voltammetry on monolayers adsorbed on Hg may reveal other properties of lipid membranes that are not evident with other experimental model systems and methodologies.

For this purpose we have studied the phase transition of DMPC monolayers spread on the surface of a hanging drop mercury electrode (HDME) following the potentiodynamic response as a function of temperature.

Our aim is to point out that in the same way by which the correspondence between monolayers on air–water interface was found with bilayers, the properties of a monolayer of DMPC on Hg show also a correspondence with monolayers in the air–water interface and with bilayers, when the potentiodynamic response was measured as a function of temperature.

In addition, the potentiodynamic response of monolayers of mixtures dimirystoylphosphatidylcholine/dimirystoylphosphatidylethanolamine at the mercury–solution interface was compared with the dipole potential of monolayers of the same composition at the air–water interfaces.

2. Materials and methods

Dimirystoylphosphatidylcholine (DMPC) and dimirystoylphosphatidylethanolamine (DMPE), from Avanti Polar Lipids Inc., were used to prepare stock solutions of 2 mg/ml in mixtures pentane/chloroform (Merck). The purity of the solvents was checked by measuring the double-layer capacitance of the mercury surface after being lowered through the aqueous solution surface on

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