Journal of Colloid and Interface Science 464 (2016) 264–276

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Surface interactions, thermodynamics and topography of binary monolayers of Insulin with dipalmitoylphosphatidylcholine and 1-palmitoyl-2-oleoylphosphatidylcholine at the air/water interface

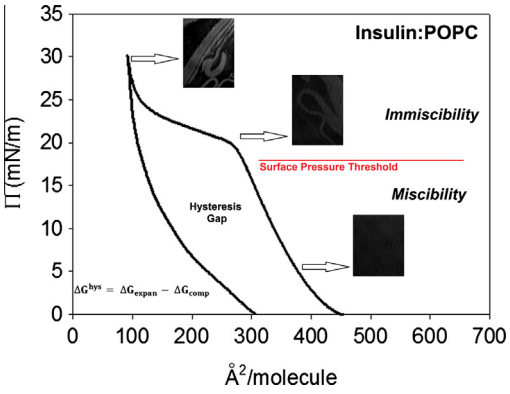
E.J. Grasso *, R.G. Oliveira, B. Maggio

CIQUIBIC, Universidad Nacional de Córdoba–CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina

HIGHLIGHTS

- Insulin forms non-ideal, stable films with DPPC and POPC.
- Under compression the films exhibit a viscoelastic or kinetically trapped organization.
- Hysteresis of films under expansion occurs with entropic–enthalpic compensations.
- BAM reveals domain coexistence at relatively high Π showing a striped appearance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 September 2015
Revised 15 November 2015
Accepted 17 November 2015
Available online 19 November 2015

Keywords:
Langmuir monolayers
Insulin
Dipalmitoylphosphatidylcholine (DPPC)
1-Palmitoyl-2-oleoylphosphatidylcholine (POPC)
Binary monolayers
Insulin surface behavior
Hysteresis

ABSTRACT

The molecular packing, thermodynamics and surface topography of binary Langmuir monolayers of Insulin and DPPC (dipalmitoylphosphatidylcholine) or POCP (1-palmitoyl-2-oleoylphosphatidylcholine) at the air/water interface on Zn^{2+} containing solutions were studied. Miscibility and interactions were ascertained by the variation of surface pressure-mean molecular area isotherms, surface compressional modulus and surface (dipole) potential with the film composition. Brewster Angle Microscopy was used to visualize the surface topography of the monolayers. Below 20 mN/m Insulin forms stable homogenous films with DPPC and POPC at all mole fractions studied (except for films with $X_{INS} = 0.05$ at 10 mN/m where domain coexistence was observed). Above 20 mN/m, a segregation process between mixed phases occurred in all monolayers without squeezing out of individual components. Under compression the films exhibit formation of a viscoelastic or kinetically trapped organization leading to considerable composition-dependent hysteresis under expansion that occurs with entropic–enthalpic compensation. The spontaneously unfavorable interactions of Insulin with DPPC are driven by favorable enthalpy that is overcome by unfavorable entropic ordering; in films with POPC both the enthalpic and entropic effects are unfavorable. The surface topography reveals domain coexistence at relatively high pressure showing a striped appearance. The interactions of Insulin with two major membrane phospholipids induces composition-dependent and long-range changes of the surface organization that ought to be considered in the context of the information-transducing capabilities of the hormone for cell functioning.

* Corresponding author at: Laboratorio de Bioquímica y Biología Reproductiva, ICTA–Facultad de Ciencias Exactas, Físicas y Naturales, e IIBYT, CONICET–Universidad Nacional de Córdoba, Av. Velez Sarsfield 1611, CP: 5016 Córdoba, Argentina.
E-mail address: ejgrasso@conicet.gov.ar (E.J. Grasso).

http://dx.doi.org/10.1016/j.jcis.2015.11.034
0021-9797/

1. Introduction

Insulin is a polypeptide hormone (MW ~5800 Da) composed of two peptide chains linked by disulfide bridges differing in their hydrophobicity and charge at physiological pH [6,29]. The hormone is synthesized and stored in β cells of the pancreas as a biologically inactive Zn^{2+}-linked hexamer [10]. When released into the bloodstream, the hexamers dissociates into dimers and subsequently into biologically active monomers [15] facilitating glucose transport into cells by at least two different steps: binding to a membrane receptor followed by activation of a glucose transporter [14,22,34]. The Insulin monomer is unstable and tends to macroscopically aggregate in aqueous solutions during storage [16,13,6]. This is a consequence of solid fibers formed by aggregation of monomers and dimers under low pH conditions [32,30]; and causes loss of hormone biological activity which is a major obstacle for developing long-term delivery formulations. Though the mechanism of aggregation of Insulin still remains unclear, some studies suggested that the presence of different hydrophobic environments (i.e. air/water or lipid/water interfaces) may be involved in the formation of aggregates [23] that may avoid gastrointestinal peptidases [11]. In any way, surface interactions will be of importance in any effect derived from aggregation or interactions of the hormone with cell membranes.

Insulin Langmuir monolayers at the air/water interface were previously described under different experimental conditions such as pH, temperature and ion concentration [29,32,24]. Also, it has been recently shown that the presence of Zn^{2+} has a profound effect on the surface behavior of Insulin monolayers [32,24,20]. We recently described the rheological properties of regular Insulin and aspart Insulin in presence on Zn^{2+}. By oscillatory compression–expansion cycles, we observed in all Insulin monolayers the development of a dilatational response to the surface perturbation, exhibiting a well-defined shear moduli in the presence of Zn^{2+}, which was higher for regular Insulin compared to aspart Insulin. Development of a shear modulus indicates behavior resembling a nominal solid, suggesting formation of viscoelastic networks at the surface [20].

Besides the receptor-mediated function of the hormone, it is important to understand its possible effects on cell membranes. The works by Pérez-López et al. using natural phospholipid mixtures represents a pioneering step in that regard [31,32]. These authors have described the behavior of binary monolayers of Insulin–sphingomyelin and Insulin–egg phosphatidylcholine (PC) at the air/water interface on pure water, NaOH and phosphate-buffered solutions of pH 7.4, and on Zn^{2+}-containing solutions [32,31]. Their results indicated that intermolecular interactions between Insulin, sphingomyelin and egg-PC depend on both the monolayer state and the structural characteristics of Insulin at the interface, which are strongly influenced by the subphase pH and salt content.

The natural phospholipids used in those studies consist of heterogeneous mixtures of many phospholipid species, each of them possibly having different interactions with the protein, while the molecular interactions of Insulin with well defined, single species of phospholipids has not, as far we know, been explored. Comprehension of specific molecular interactions of Insulin with defined phospholipids, and their longer-range consequences on the surface organization is necessary in order to further understanding of such effects. With this aim, we studied in this work the surface behavior of mixed films of Insulin with DPPC and POPC, two major and well characterized constituents of egg and natural PCs, on Zn^{2+} containing solutions (where Insulin molecules form hexamers showing well defined organization states and interesting viscoelastic properties [29,24,20]). Besides classical miscibility studies, we focused on the thermodynamics of the mixing process, on the presence of monolayer hysteresis and on exploring the surface topography of the films by Brewster Angle Microscopy. Our results reveal novel features of the surface organization and thermodynamics of these binary interfaces that may also have some implications for the stability of possible formulations and for the construction of nanofilms as supports for stimulated cellular growth. In this connection, we have previously shown that surfaces coated with Insulin, in presence of Zn^{2+}, selectively influence hippocampal neuron polarization depending on the molecular organization of the Insulin film on which cells are grown; this indicated that recognition events mediated by different molecular organizations of an Insulin-coated surface can finely mediate and modulate neuronal differentiation [21].

2. Materials and methods

2.1. Reagents

Bovine Insulin (MW 5733 Da) was purchased from Sigma–Aldrich, St Louis, MO, USA. DPPC and POCP were purchased from Avanti Polar Lipids, USA. Aqueous subphases were prepared with ultrapure water produced by a Millipore water purification system. NaCl and $ZnCl_2$ were provided by Merck (Darmstadt-Germany). The surface tension and resistivity of the ultra-pure water used were 18.2 MΩ cm and 72.2 mN/m at 24 °C, respectively.

2.2. Insulin-DPPC/POPC binary monolayers

2.2.1. Compression isotherms

Absence of surface active impurities before spreading the monolayers or in the spreading solvents was routinely checked [21] by reducing the initial trough area to about 10% of the initial area in the absence of spread Insulin-DPPC or POPC or by spreading 50 μL of pure solvents; the changes in surface pressure (Π) and surface potential were less than ±1.0 mN/m and ±30 mV, respectively. Stock solution of Insulin (14 mg/mL) were dissolved in ultrapure water −0.06 M HCl (pH = 2.5) [29]. DPPC or POPC were dissolved in chloroform:methanol (2:1). Spreading solutions of Insulin (0.125 nmol/μL) were freshly prepared daily by adding the required amount of Insulin in water solution to the solvent solution (chloroform:methanol, 2:1 v/v) so that the proportions reached the ratio chloroform:methanol:water (60:30:4.5 v/v/v) which allows the system to remain in a single homogenous phase [18]; compression isotherms of Insulin spread from water [21] or from fresh solvent solutions were indistinguishable. Langmuir monolayers were formed onto the aqueous subphase (NaCl 145 mM plus $ZnCl_2$ 1 mM, pH 6.3) by spreading 25 μL of Insulin–lipid premixed solvent solutions (0.16–1.11 nmol/μL) in the desired proportions. The protein and lipid concentrations were adjusted so that mixing of aqueous and solvent solutions were always kept within the proportions maintaining the final solution in a single homogeneous phase [18]. We waited 10 min for solvent evaporation and monolayer equilibration before compression. Isometric compression and decompression isotherms (speed = 20 $Å^2$/molecule/min), were carried out in a KSV-minitrough, having a Teflon trough with a surface area of 266 cm^2 and a Wilhelmy-Pt plate surface pressure sensor and two symmetrically moving barriers. The temperature was maintained at 24 ± 0.5 °C with an external circulating water bath (Haake F3C). The collapse pressure, surface pressure point for molecular reorganization and limiting mean molecular area of the Insulin films were determined from the third derivative of the compression isotherms [8], after being reproduced in at least three independent experiments.