AFM CHARACTERIZATION OF SUPPORTED PLANAR BILAYERS OF THE INNER MEMBRANE OF *ESCHERICHIA COLI*

Laura Picas[1], *M. Teresa Montero*[1,±], *Antoni Morros*[2,¥], *Gerard Oncins*[3], *Jordi Hernández-Borrell*[1,±,*]

[1]*Departament de Fisicoquímica, Facultat de Farmàcia UB, 08028-Barcelona.* [2]*Unitat de Biofísica, Departament de Bioquímica i Biología Molecular, Facultat de Medicina, UAB, 08193-Bellaterra (Barcelona), Spain.*[3] *Serveis Científico Tècnics UB, 08028-Barcelona, Spain. Contact@E-mail: jordihernandezborrellb.edu*

One of the most intriguing questions underway in present membrane biology research is the understanding of the interactions between phospholipids and transmembrane proteins (TMPs). Among TMPs, lactose permease (Lac Y) of *E. coli* is considered as a paradigm for the secondary transport[1]. Although the energetic behind the conformational changes underwent by TMPs, i.e. during lactose-H^+ cotransport[2], is not totally understood, it is conceivable that the work required to induce a transition between two states may arise from changes in the lateral pressure exerted by the phospholipids onto protein surface[3,4] . Hence, phospholipids species closest to the protein should have the adequate physicochemical properties, compressibility and fluidity among them, to adapt to transversal domains of TMPs[5,6]. On the other hand, the natural occurring phospholipids in biological membranes belong to the heteroacid type, with one acyl chain saturated at the *sn*-1 and the other one unsaturated at the *sn*-2 position linked to the glycerol backbone[7]. These phospholipids, 16-18 C long, are in fluid phase in physiological conditions and are highly compressible. Such is the case with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) the most abundant phospholipid of the inner membrane of *Escherichia coli*. In regard to the interplay with membrane phospholipids it has been demonstrated that LacY is dependent on phosphatidylethanolamine (PE) presence for *in vivo* function and also for its correct folding [8,9]. Actually, LacY has been reconstituted in a functional state only in phospholipid matrices with high levels of PE, either in the native *E. coli* polar phospholipid membrane extracts [10,11] or in binary mixtures of phosphatidylgycerol (PG) and phosphatidylethanolamine (PE)[12,13]. For this reason, it is reasonable to assume that PE should be part of the neighboring phospholipids that surround the protein, namely the annular region. On the other hand, physical properties of POPE, as its condensation in monolayers[4] at the air water interface and its prone-non bilayer tendency, should play a definite role in providing the adequate lateral surface pressure and membrane curvature for functional packing of the protein[4,6].

The aim of the present work is to study by means of AFM how POPE asymmetric bilayer deposited on mica respond to temperature. In suspension, POPE shows two well characterized thermal transitions: (i) the solid-crystalline (L_β) to the liquid-crystalline (L_α) at ~24 ºC, also known as the gel to liquid phase transition temperature (T_m); and (ii) the lamellar to inverted hexagonal (H_{II}) phase transition at elevated temperatures, ~80 ºC (T_H)[14].

[±] Members of the Institut de Nanociència i Nanotecnologia de la Universitat de Barcelona (IN^{2}UB) and [¥]Centre d'Estudis en Biofísica (CEB) de la UAB.

* Corresponding author: jordihernandezborrell@ub.edu

The thermal behavior and the existence of a single or double transition temperature are of interest to unveil the nature of the POPE phase transitions in supported planar bilayers. In the present work, we applied force spectroscopy (FS) to study the load needed to puncture a POPE bilayer as a function of the temperature. This range of temperatures comprises the transition from L_β to L_α and L_α to H_{II} phases in bilayers.

References:

[1] Guan, L. and Kaback, R., *Annu. Rev. Biophys. Struct*. **2006**.
[2] Yin, Y.; Jensen, M.O.; Tajkhordish, E.; Schulten, K. *Biophys., J.* **2006**.
[3] Cantor, R.S., *J. Phys. Chem.B.* **1997**.
[4] Domènech, Ò.; Ignès, J.; Montero, M.T., Hernández-Borrell, J., *.Phys.Chem.B*.**2007**.
[5] Bezrukov, S. M., *Curr. Opin Coll. & Interface Sci*. **2000**.
[6] Van den Brink, E.; Killian, J. A.;de Kruijff, B. Biochim. Biophys. Acta **2004**.
[7] Dowhan, W. *Annu. Rev. Biochem*. **1997**.
[8] Bogdanov, M, Sun J, Kaback H R, Dowhan W., *J Biol Chem.* **1996**.
[9] Bogdanov, M, Dowhan W., *J Biol Chem.* **1995**.
[10] Vitanen, P., Newman, M. J., Foster, D. L., Wilson; T. H., Kaback, H. R, *Methods Enzymol.*, **1986**.
[11] Consler, T. G, Persson, B. L., Jung, H. Zen, K. H. Jung, K. Prive G. G., Verner, G. E. and Kaback, H.R., *Proc. Natl. Acad. Sci*. USA, **1993**.
[12] Le Coutre, J., Narasimham, L. R., Patel, C. K. N, and, H. R Kaback, *Proc. Natl. Acad. Sci.* USA, **1997.**
[13] Zhao; M., Zen; K. C., Hernández-Borrell, J.; Altenback, C.; Hubbell; W. L, Kaback, H. R. *Biochemistry*, **1999**.
[14] Epand, R.M.; Bottega,R. *Biochim. Biophys. Acta* **1988**.

Figures:

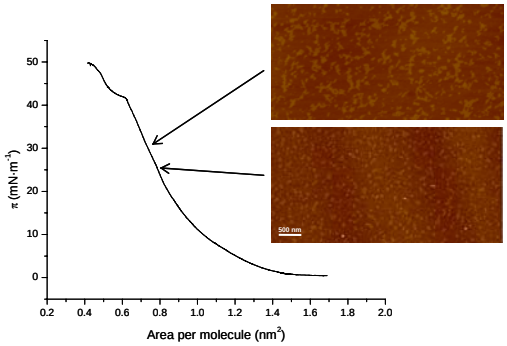

Figure 1. AFM images of POPE monolayers acquired in air

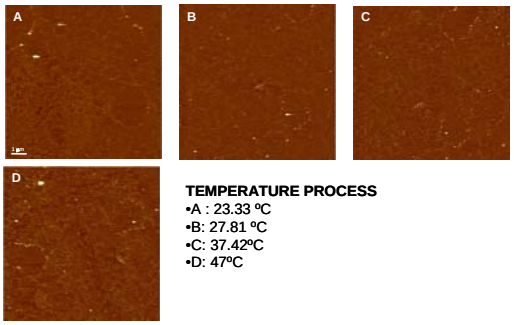

Figure 2. AFM images of asymmetric POPE bilayers acquired in liquid at different temperatures.