TOWARDS INSERTION OF A TRANSMEMBRANE PROTEIN INTO SUPPORTED PLANAR BILAYERS: PRELIMINARY STUDIES

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A real paradigm or model for the secondary transport mechanism in biomembranes is the lactose permease (LacY) of *Escherichia coli* [1], a well characterized transmembrane protein that catalyzes the coupled stoichiometric translocation of β-galactosides and H^+ across the cytoplasmic membrane. Integral membrane proteins are surrounded by a layer of phospholipids known as annular lipids, which composition and physical properties play a role in the function of the protein [2]. In physical terms, the boundary region should provide the adequate thickness to embed the protein. Basically the length of the hydrophobic domains of the protein (12 spanning alfa helices for LacY) should match the length of the lipid bilayer membrane [3]. In regard to the interplay with membrane phospholipid it has been demonstrated that the protein is dependent on phosphatidylethanolamine (PE) presence for *in vivo* function and also for its correct folding [4,5]. Indeed, 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 or in binary mixtures of phosphatidylgycerol (PG) and PE.

The aims of this work are two fold: (i) to investigate if there is preferential selectivity of LacY for PE and PG; and (ii) to reconstitute LacY into planar bilayers formed with PE and PG in order to visualize the annular region of the protein.

FRET experiments between a Lac Y single tryptophan mutant (w151/C154G) [6] and pyrene phospholipid derivatives were carried out to obtain information on the annular lipid composition of LacY from *E. coli*. We investigate, qualitatively, the presence of either PG or PE within the shell of adjacent boundary lipids. Thus, two pyrene derivates were used as acceptors: 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-Glycero-3-phosphoglycerol (PPDPG) and 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-Glycero-3-phosphoethanolamine (PPDPE). The W151 mutant reconstituted in proteoliposomes of POPE:POPG (3:1, mol:mol) was used as the donor.

In Figure 1 the variation of the energy transfer efficiency ($ET=1-I/I_0$) versus temperature, for PPDPE (solid line) and PPDPG (doted line) (left), and the model for the process between the single tryptophan protein and the pyrene derivates (right) are shown. PPDPE shows higher values of *ET* than PPDPG in all range of temperatures studied. On the other hand, the efficiency for PPDPG increases progressively as the temperature increases. These results indicate, firstly, an enrichment in PE at the vicinity of the protein, and secondly, a higher lateral mobility of PG. Assuming a single pair, the distance between the donor and the acceptor can be estimated according: $ET = R_0^6 / R_0^6 + r^6$. The *r* values for PPDPE and PPDPG were 2.6 and 2.9 nm respectively, suggesting that the first shell of the annular region may be mainly formed by PE.

Incorporation attempts of LacY into supported planar bilayers (SPBs) were performed by incubation of purified LacY in dodecylmaltoside (DDM) detergent with planar bilayers of POPE:POPG (3:1, mol:mol) previously formed and extracting the excess of detergent [7].

The effect of the detergent and Ca^{2+} presence on the stability of the SPBs was studied through AFM *in situ* observations. Figure 2 shows the effect on the bilayer of a DDM solution at the CMC in absence (2A) and presence (2B) of LacY (20 mg/ml) after 15 minutes of incubation at room temperature. Some bright spots can be seen in the SPB in presence of protein (Fig 2B). Single entities were not observed suggesting that Lac Y may be aggregated in some extension (inset in image 2B). Work is underway to unveal the annular region.

References:

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Figure 1

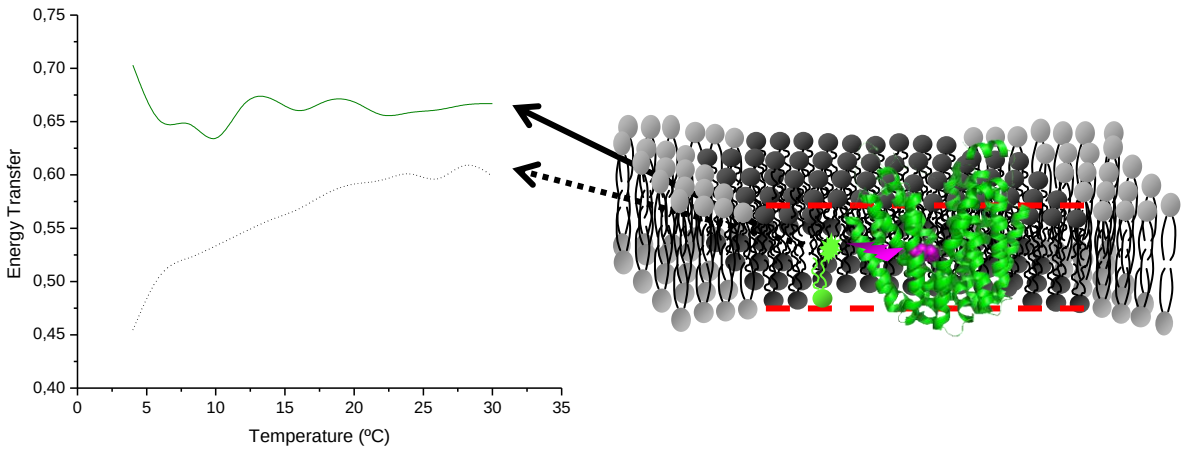

Figure 2