EFFECT OF DIVALENT CATIONS ON PORIN INCORPORATION IN PLANAR BLM

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Biological membranes contain as a basic structural unit a phospholipid bilayer.
Phospholipid membranes in the form of bilayer films (BLM) are currently used as experimental
models for transport phenomena of the biological membranes. In particular, bilayers made of acidic
phospholipids, present the advantage to investigate the bioelectrical phenomena across the
biological membranes.
This class of phospholipids by having a fixed charge might bind ions that play an important role on
many physiological processes. It is known that Ca\(^{++}\) and other alkaline cations change the
electrostatic potential of the negatively charged bilayer membranes either absorbing to the
phospholipids or accumulating in the aqueous diffuse double layer, and this potential change may
induce conductance variation (1).
Cd\(^{++}\) another divalent cation has been shown to exert influence on the permeability of some
anaelectrolytes across BLM made of negatively charged phospholipids, but not on the zwitterionic
phospholipids (2).
We have studied the effect of divalent cations as Ca\(^{++}\) and Ba\(^{++}\) that exert a cross-linking together
with a screening effect respectively, on the kinetics of incorporation of the mithocondrial porin in
bimolecular lipid membranes made with phosphatydilinositol.
In order to understand how lipids of the bilayer affect protein incorporation at the molecular level
we must consider that protein crossing the bilayer has to surmount different regions caraterized by
different properties as interfacial region, head groups, lipid backbone and hydrocarbon core.
In this study we focused our attention to the role of the interface and head group regions of the
BLM in the porin incorporation.
The artificial membranes were made of phosphatidylinositol chromatographically pure in n-decane (1% w/w). The bathing media were KCl 1M and KCl 1M plus CaCl₂ or BaCl₂ 10 mM. Porin was added at membrane "black" on the two aqueous bathing solutions. Bilayers conductivity and capacitance measurements were carried out at temperature of 25 ± 2 °C and studied by means of alternate current (f = 1 Hz) recording the voltage after a current to voltage converter in series with the membrane; simultaneously electrical capacitance was measured at 1 KHz before and during porin incorporation (3).

We noticed that when the membranes are in the presence of Ca⁺⁺ or Ba⁺⁺ there was an exceptional stability.

With the different bathing solutions no significatively differences were observed in membrane conductance values whereas small capacitance variations were recorded.

Once the membrane has became black porin, at a fixed concentration, was added to the aqueous solutions, and after a lag time due to the diffusion of the protein in the solutions and of the time to overcome the membrane barriers, an increase of the voltage output due to the channels formation was recoded. This phenomenon shows a "S-shaped" kinetics. In contrast to the kinetics of incorporation of the porin in oxidized cholesterol lipid membranes (4), showing an hyperbolic shape. These different kinetics may be explained by the different interface barrier; in fact when the experiment were conducted with porin already present in the bathing solutions before membrane formation, the different kinetics persisted in the phosphatidylinositol and in oxidized cholesterol BLM.

The S-shaped kinetics persistes also when porin is incorporated in phosphatidylinositol membranes in the presence of Ca⁺⁺ or Ba⁺⁺; but the curve in presence of the Ca⁺⁺ is shifted to the right. This shift is much more pronounced in the presence of Ba⁺⁺.

It is known that Ca⁺⁺ affects the surface potential of BLM containing negatively charged lipids. The protein incorporation into BLM can be regarded as a two steps process: I) the interaction at the surface barrier, and II) the penetration into hydrocarbon region.

In the first step the protein encounter an higher resistance in phosphatidylinositol membrane because it has to overcome the interface region consisting of a diffuse double layer, the membrane surface and the polar head groups.
Ca++, by exerting a cross-linking and screening effect on negatively charged membranes, retards the porin incorporation.

At the moment we are not able to distinguish which of the two effect is prevalent; but results in presence of Ba++, that exerts a screening effect only seem to indicate that the latter effect is prevalent. In order to establish the pure cross-linking effect experiments are in progress.

The influence of the divalent cations on the protein incorporation into BLM, that are sensitive to changes in environmental conditions, make this study appealing in order to provide a better knowledge in the molecular mechanism of the protein incorporation and channel formation.

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