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Session 10

LIPID MEMBRANE VESICLES AND MICROCAPSULES

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PORE FORMATION IN LIPID MEMBRANES

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Porin molecules form large water filled pores through the hydrophobic core of the outer membrane of gram-negative bacteria and mitochondria. These channels act selectively on the permeation of hydrophilic solutes.
Pore forming properties can be studied in reconstitution experiments with planar lipid bilayers and liposomes.
Porin addition to the bathing solutions of a planar lipid bilayer membrane results in the formation of ion permeable channels.
Aim of this research was the analysis of pore incorporation kinetics in different experimental conditions and studied by means of sinusoidal current.

The artificial membranes were formed, with phosphatidylinositol (PI; 1%) in n-decane, brushing the solution on a small circular hole (diameter 1.3 mm) of a teflon cell with compartments of 4 ml volume.
PI was extracted from ox brain and purity chromatographically tested.
Porins were purified by mitochondria from different tissues of mammalia.
Ionic solutions were KCl 0.5 or 1M. The effect of Ca on the kinetics of protein incorporation was tested adding $CaCl_2$ 1-10 mM to both phases.
Aqueous solutions were used unbuffered; the pH was approximatively 6.5.
By means of two Pt electrodes in bathing solutions the membrane was connected in series with a current to voltage converter (measurement device schematically represented by a parallel resistance and capacitance) and the sinusoidal input voltage ($VS = V_s \sin \omega t$; $f = 1$ Hz) was applied to the whole series.
Voltage V_1 at the converter was directly related to the membrane current.
The electrical capacitance of the system was measured at 1 kHz before adding porin and then followed at some times during incorporation. The experiments were performed at room temperature (22-25°C), but in any single experiment the temperature did not vary more than 0.5°C.

After addition of porin from a stock solution to the solutions bathing a completely black membrane the transmembrane electrical current increased with time, indicating protein fusion and pore formation into the lipid matrix (Fig. 1A).
The time-course of membrane current was S-shaped. Similar increasing membrane currents were observed irrespective whether the porin was added to the aqueous solution on one side of the membrane only or to both solutions.

After many minutes, depending on porin concentration, a steady state membrane current value was reached (Fig. 1).
At large concentrations of the porin the membrane current quickly rises to values many order of magnitude the level with untreated membrane.
In some experiments a stationary current level could not be reached and the current increased continously until membrane breakage.
Porin incorporation rate decreased lowering porin concentration (Fig. 1). A delay in channel formation and a slower kinetics were obtained when porin was already present in aqueous solutions prior to the formation of the membrane (Fig. 2B).
During the membrane blackening period it seems more difficult for porin molecules to diffuse into the membrane and to form channels. Probably the process of bilayer thinning constitute a limiting factor for protein molecules to assume conformation which allows for the pore formation.
In control conditions (KCl 1M or 0.5M) transmembrane current was calculated, because of the phase angle, considering the variations of membrane electrical resistance and capacitance during porin incorporation.
To obtain analytical time derivative of electrical potential, V_1, the experimental data were fitted with a four-parameter logistic equation. The kinetics of pore formation depends on the applied voltage.
For a same porin concentration in bathing solutions membrane current increased more rapidly at higher voltages applied (Fig. 3). The presence of $CaCl_2$ reduces the dependence on the voltage (Fig. 4B) and with $CaCl_2$ 10 mM, at low voltages, the membrane current values, after a time lag, were lower than with $CaCl_2$ 1 mM.

The pore dependence of membrane electrical conductance and capacitance has been approached with simple geometrical considerations and single channel characteristics. Pores made of porin are quite large, eventually formed by the cooperation of three monomers, and then relatively open so that the pore water can maintain a structure closer to that of bulk water and the aqueous phases are probably dielectrically quite similar. Whit this hypothesis a linear decreasing of capacitance and a non linear decreasing of membrane resistance result increasing the number of pores.
Analytical time dependence of pore formation was introduced describing the phenomenon of channel formation as resulting from diffusion of porin molecules from the bathing solutions into the lipid membrane (a function with exponential terms was obtained) or with the above mentioned fitting procedure.
During pore formation the current depends not only on the membrane resistance and capacitance but also on the time derivative of the capacitance.

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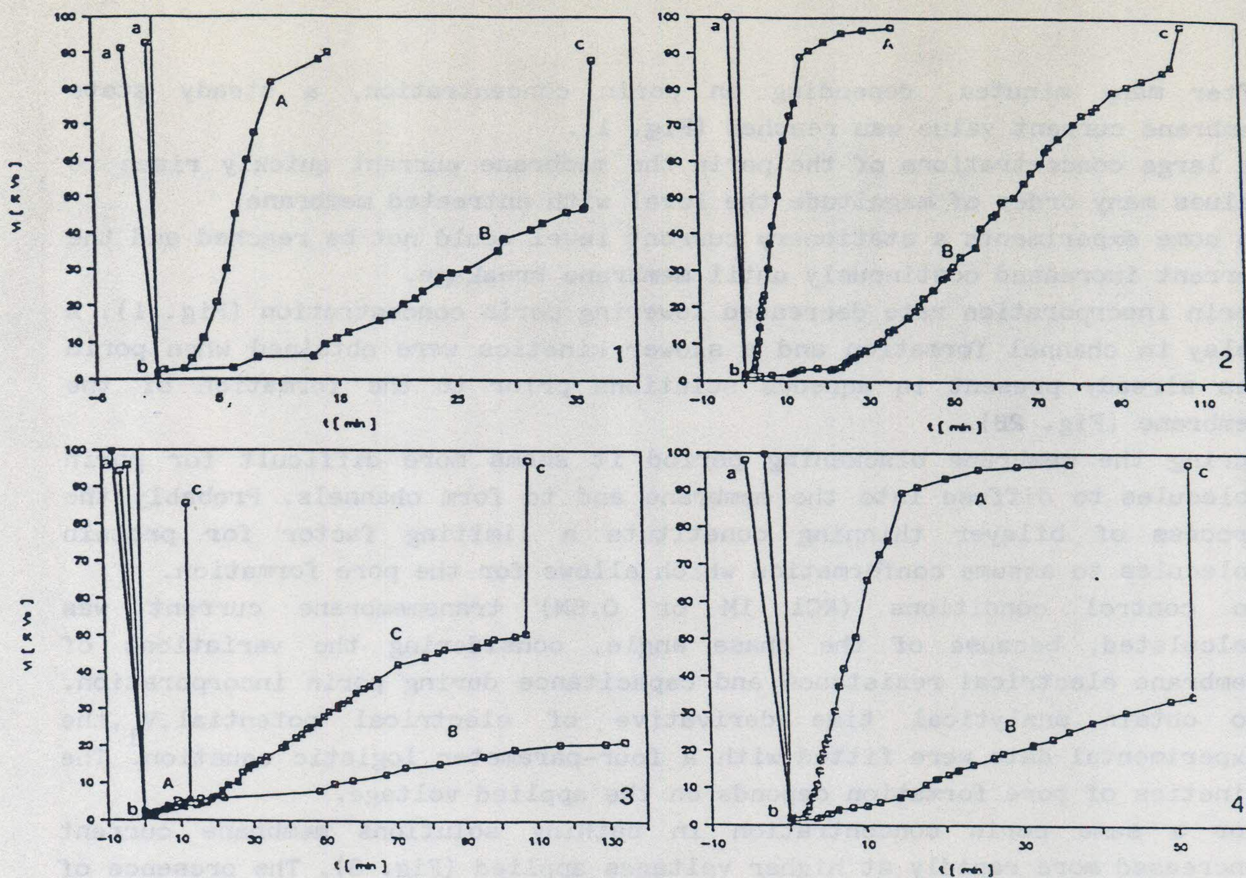

Maximum value of the voltage at the measurement device (V_l = % V_s) as a function of time t after the addition (at t=0) to both sides of the PI membrane (completely black) of porin 602 ng/ml (1A) and 72 ng/ml (1B), final concentrations in the bath; V_s = 8.3 mV.
2) Porin 48 ng/ml, V_s = 33.0 mV; in 2B porin was already present in aqueous solutions (at t=0) prior to the formation of the membrane.
3) Porin 96 ng/ml ; V_s = 8.3 mV (3A,B), 33.0 mV (3C).
4) Porin 48 ng/ml, V_s = 33.0 mV ; $CaCl_2$ 1mM was in bathing solutions (4B). Ionic solutions were KCl 1M ; V_l values with bathing solutions but without membrane (a), with the membrane (b) and after membrane breakage (c) are reported. Temperature 25 ± 0.5 °C ; frequency 1 Hz.