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ION CONDUCTING PORES INDUCED BY OLIGO-L-ALANINE

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In connection with the ion permeation ability of peptides, samples of $HCO-(Ala)_n-OCH_3$ with n = 20 and 24 were shown to induce single channel events in BLM. However, as a water induced α to β transconformation occurs, the question of the conformation of the active species and of the pore structure is still open.

Except for Gramicidin A for which it seems well established that the ionic channel is based on a π_{DL} channelled helical structure, polypeptidic conformations involved in the formation of pores by proteins is still obscure although several models have been proposed. An important feature of one kind of these models is the presence of glycyl residues which were expected to adopt conformational states of D residues and thus allow the building up of intramolecular helical structure acting as channels (1,2). However, this hypothesis has never been confirmed by any conformational investigation which, in contrast, suggests an α-like helical structure for the pore forming polypeptides $(Leu-Ser-Leu-Gly)_n$, $(Ser-Leu-Gly)_n$ and their O-benzyl derivatives (3). Therefore, as the proposed models can be rejected and as the role of the glycyl residues is questioned, we were led to investigate the possibility of pore formation by very simple polypeptides such as poly-L-alanine.

Four samples with the general formula $HCO-(Ala)_n-OCH_3$ with n = 10 (I), 15 (II), 20 (III) and 24 (IV) were prepared by the solid phase method and we report here some preliminary observations made with these simple polypeptides when added to black lipid membranes.

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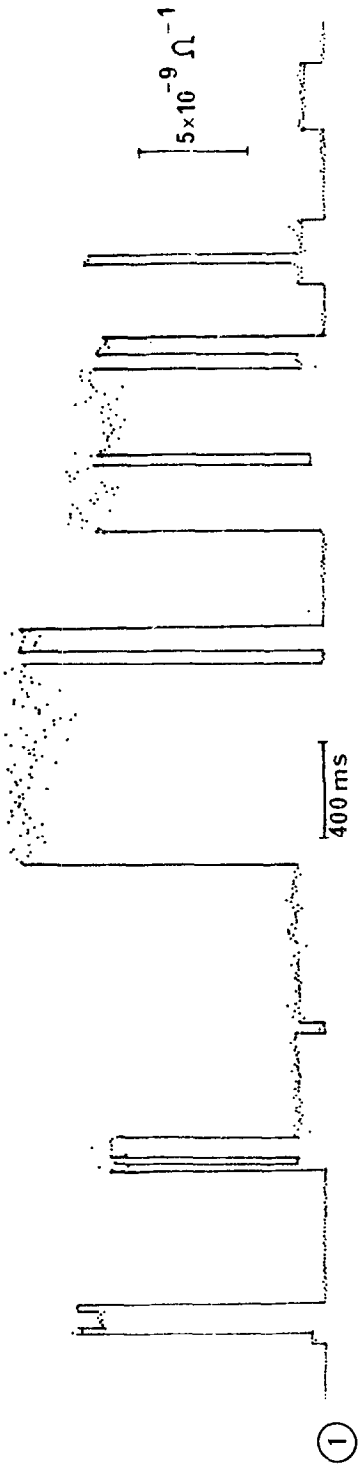
BLM were prepared as already described (3) from 2 % solutions of glyceryl monooleate or dioleoylphosphatidylcholine in decane. For solubility reasons the polypeptides were added either with the lipids in the membrane forming solution or through the aqueous phase in hexafluoro-2-propanol-trifluoroethanol solutions for single channel experiments. In order to check the effect of these polypeptides on the permeability of bimolecular membranes, current fluctuations due to discrete changes in conductance and steady state conductance measurements were performed with these membranes.

While no current fluctuations could be detected for sample I and very rare events for sample II, discrete fluctuations are easily observable for both samples III and IV suggesting that they are able to create ion conducting units when incorporated into BLM. The wide range of pore conductances observed (Fig. 1) and the fact that they are not selective for monovalent alkali ions (Fig. 2) is strongly reminiscent of the behavior already described for the polypeptide $(Leu\text{-}Ser\text{-}Leu\text{-}Gly)_n$ (3) and suggest that the pores may be built by assemblies of several molecules.

The steady state conductance measurements are also in accordance with these observations. The conductance is increased more than 100 times with samples III and IV when added in the membrane forming solution as compared with the conductance of the undopped membrane ($10^{-7}\ \Omega^{-1}.cm^{-2}$) while no effect is observed with sample I nor with sample II.

The conformational structure of these polypeptides was investigated. For samples III and IV two conformational states can be considered, i.e., the α-helix and the β-folded structure. From infrared spectroscopy data, both samples undergo in the solid state a water induced α to β transition when brought to contact with water molecules. This behavior suggests that the incorporation of oligo-alanine to lipid membranes which occurs from an aqueous interfacial environment can also favour the β form. This is confirmed by the circular dichroism spectrum of sample III containing vesicles made from lipid which shows a minimum at 217 nm. However, as already suggested for the antibiotic alamethi-



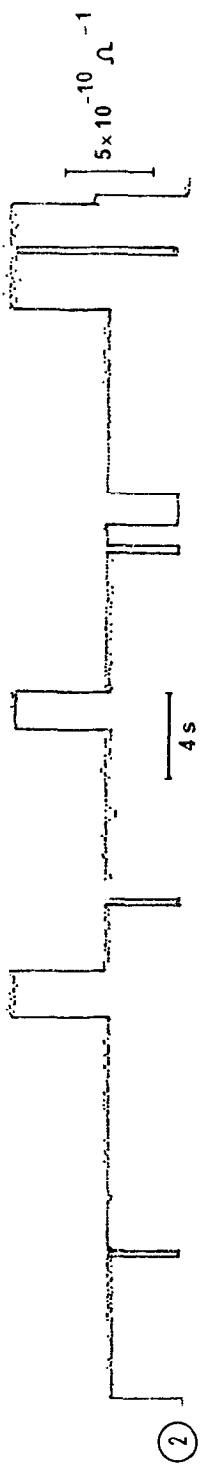


Figure 1 - Fluctuations of the transmembrane current in presence of small amounts of sample III in 1 M KCl.

Figure 2 - As in figure 1 in 0.5 M $CaCl_2$.

cin (4), an electrical field induced α conformation may explain the pore formation observed with such a system. The importance of the α conformation seems to be confirmed according to the behavior of a poly-L-alanine sample which does not undergo an α to β transition and which is also able, but with difficulty owing to its high molecular weight, to form pores in membrane models. The same is true for poly-α-amino isobutyric acid which shows also pore conductance fluctuations but cannot exist in the usual β conformation (5). However the question of the conformation of the active species is still open.

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