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IONIC CHANNELS OF SOME GLYCINE-RICH SYNTHETIC POLYPEPTIDES

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The ion transport properties of glycine-rich synthetic polypeptides (Ser-Leu-Gly)$_n$, (Leu-Ser-Leu-Gly)$_n$ and their O-benzyl derivatives have been examined. When incorporated into lipid bilayers membranes, all these model molecules give rise to ion permeation through single channel events. Their behavior, peculiarly that of the O-protected derivatives rules out the channeled model previously proposed for (Leu-Ser-Leu-Gly)$_n$ in which the hydroxyl groups of the seryl residues were supposed to play a role in the ion translocation process. This latter polypeptide also permeates membranes to calcium. Preliminary investigations suggest an α-like helical conformation rather than a β helix with an intramolecular channel.

INTRODUCTION

Ionic exchanges through cell membranes are of major importance in the understanding of biological energetics. Therefore much attention has been given to transmembrane peptides and proteins which are thought to form ionic channels. Up to date most studies on channel forming peptides were devoted to linear Gramicidin as this antibiotic was considered as a model molecule for the permeabilisation of membranes (1-5). However, its biological signification is much restricted owing to its origin and to its peculiar stereochemical sequence. As to all L-polypeptides chemically closer to transmembrane proteins, possible conformations were proposed by Urry (5) and Kennedy *et al.* (6) and ion transport properties of glycine-rich synthetic peptides used as tentative model molecules have been reported (6-8), however without any experimental conformational investigation.

In order to check the proposed conformations and eventually relate them to the existence of ionic channels we have synthesized polypeptides containing a glycyl residue every 3 or 4 residues. The choice of the other amino acids was made on the basis of the work of Kennedy *et al.* (6) in order to compare our results with those previously reported. Thus we describe here the ion transport properties and some preliminary conformational studies of four polypeptides, i.e. [Leu-Ser(OBzl)-Leu-Gly]$_n$, [Ser(OBzl-Leu-Gly]$_n$ and their Ser-deprotected parent molecules.

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EXPERIMENTAL PART

Samples

The polypeptides were obtained by polycondensation in benzene of the p-nitrophenyl ester of the corresponding tri or tetrapeptide. Removal of the benzyl O-protecting groups was achieved to more than 99.5 % as shown by UV spectroscopy with HBr in hexafluoroisopropanol-chloroform mixtures.

Single channel experiments

Black lipid membranes were formed from a 2 % solution of dioleoylphosphatidylcholine (Sigma) in nonane in teflon cells filled with 1 M aqueous electrolyte solutions. The membranes areas were about 1.5×10^{-3} cm^2. A Keithley model 427 was used as current amplifier and the informations stored on a micro computer Apple II. Frequency sampling was 10 to 20 Hz. The polypeptides were added from ethanolic solutions to the aqueous phases.

RESULTS

Ion conductivity

1. Protected polypeptides

When incorporated into lipid bilayers, both protected polypeptides give rise to permeable membranes through discrete conductance fluctuations events for alkali ions (Fig. 1). As shown in table 1, both polypeptides have the same ion selectivity order than linear Gramicidin (2) and the same Ca^{++} blocking effect is observed. Also the order of magnitude of the conductance is very similar (9). It should be mentioned that less intense and less frequent fluctuations than those reported in table 1 are also observed (see Figs 1 and 2), but at this stage of the work it cannot be decided whether this reflects some heterogeneities arising from the polydispersity of the samples or different types of conducting pores.

2. Deprotected polypeptides

Like the O-protected polypeptides, addition of small amounts of $(Leu\text{-}Ser\text{-}Leu\text{-}Gly)_n$ or $(Ser\text{-}Leu\text{-}Gly)_n$ to black lipid membranes increased their conductivity. The case of the polytripeptide will not be discussed here in detail as

Table 1 - Conductances of the most frequent events for the two O-protected polypeptides in dioleoylphosphatidylcholine membranes and 1 M electrolytes.

Sample / Ion	$[Leu\text{-}Ser(OBzl)\text{-}Leu\text{-}Gly]_n$		$[Ser(OBzl)\text{-}Leu\text{-}Gly]_n$	
	$\Lambda(10^{-11}\Omega^{-1})$	$\Lambda i/\Lambda K^+$	$\Lambda(10^{-11}\Omega^{-1})$	$\Lambda i/\Lambda K^+$
Li^+	0.185	0.10	0.30	0.24
Na^+	1.10	0.59	0.98	0.78
K^+	1.85	1.00	1.25	1.00
Rb^+	3.80	2.05	3.4	2.74
K^+ 1 M, Ca^{++} 0.5 M	0.475	-	0.425	-

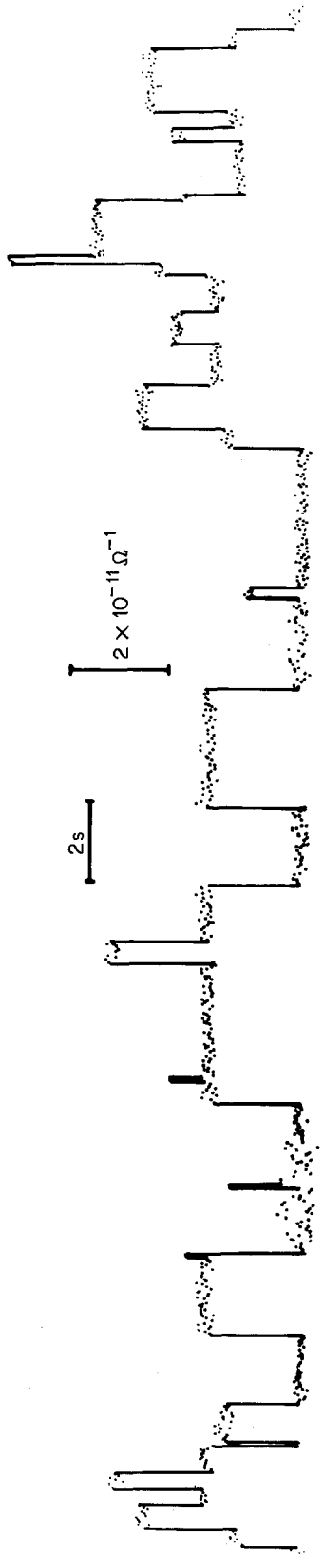

Fig. 1 - Fluctuation of the membrane current in presence of small amounts of [Leu-Ser(OBzl)-Leu-Gly]n in 1 M KCl. Applied voltage : 100 mV.

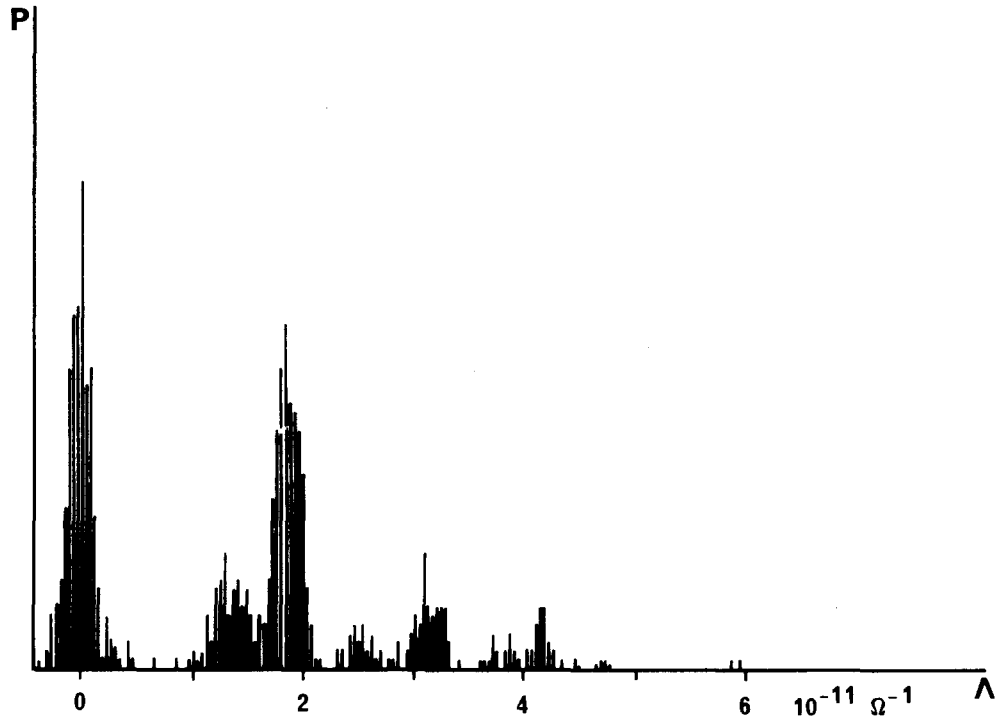

Fig. 2 - Distribution of the intensity of the transmembranes current corresponding to the trace of Fig. 1.

it induces very rapid fluctuations (lifetime less than 10 msec) of the transmembrane current which cannot be analyzed.

Concerning the polytetrapeptide, its behavior strongly differs from that of the O-benzyl analog. Indeed, a wide range of channel conductances can be detected for alkali ions (for instance 2.3×10^{-11}, 8.3×10^{-11}, 3.5×10^{-10} and 1.3×10^{-9} Ω^{-1} in 1 M KCl). It appears that beside the pores of the highest conductance (Fig. 3) which were already observed by Kennedy *et al.* (6), channels of lower conductances (Fig. 4) are also noticed. Further, an interesting point is that the pores are not specific for monovalent alkali ions as events of various conductances are also detected for calcium cations (from 3.5×10^{-12} Ω^{-1} up to 1.5×10^{-9} Ω^{-1}).

Conformational investigations

Owing to the poor orientation of films cast from chloroform or hexafluoroisopropanol, the electron and X-rays diffraction patterns of all polypeptides do not allow any definitive conclusion about their conformation. However, a helical conformation with 12 residues per turn as proposed by Kennedy *et al.* (6) can be ruled out for the protected polytetrapeptide as a hexagonal system with dimensions as small as a = 1.26 nm has been found. Spectroscopic data, i.e., circular dichroism in solution (Fig. 5) and infrared in the solid state or in chloroform solutions which show Amide I and II band at 1650 and 1550 cm^{-1}

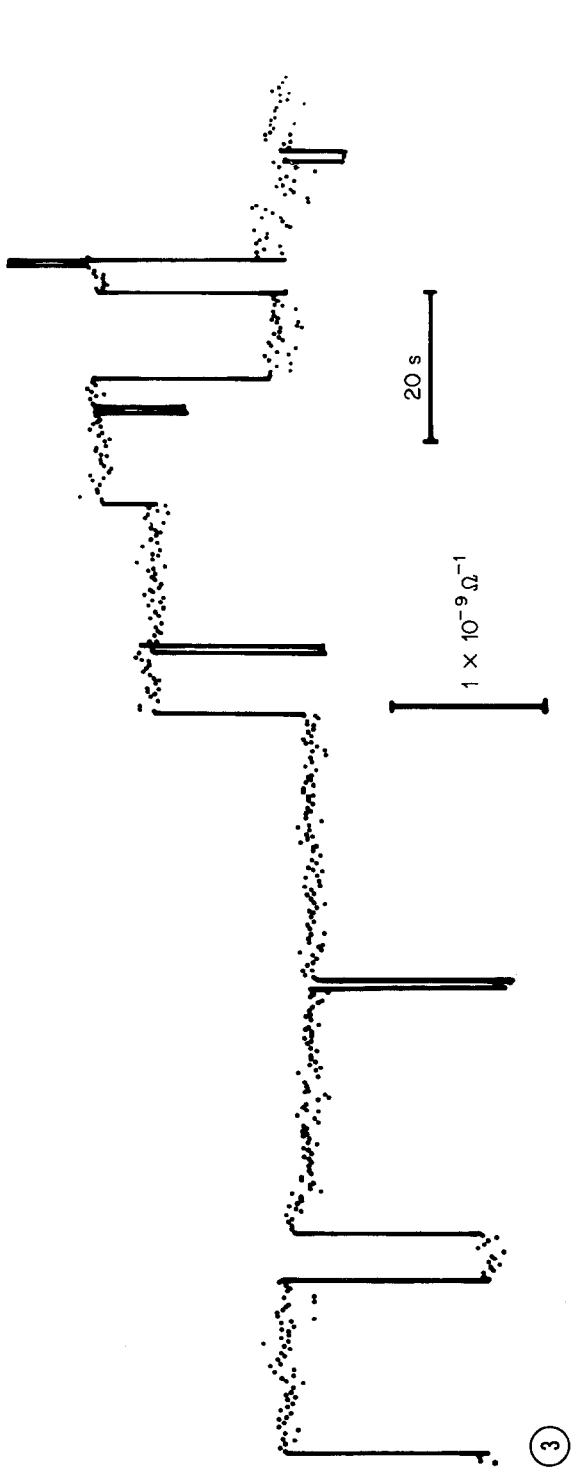

Fig. 3 - Fluctuation of the membrane current in presence of small amounts of $(Leu\text{-}Ser\text{-}Leu\text{-}Gly)_n$ in 1 M KCl. Applied voltage : 50 mV.

Fig. 4 - As for Fig. 3. Applied voltage : 100 mV.

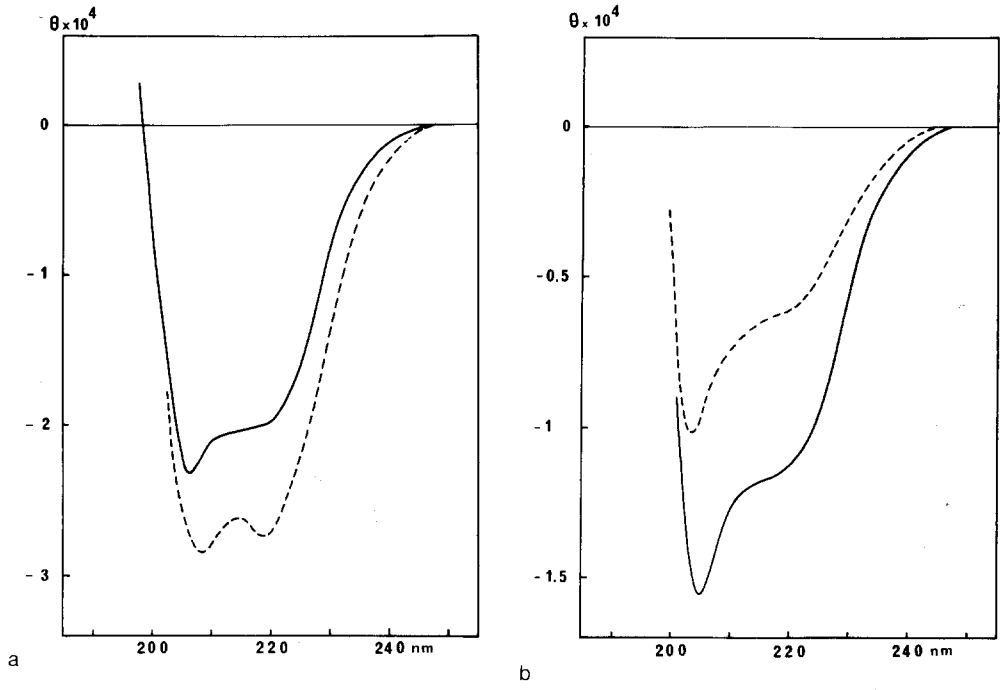

Fig. 5 - Circular dichroism spectra in hexafluoroisopropanol-trifluoroethanol (1:9) mixtures of : a) ——— $(Leu\text{-}Ser\text{-}Leu\text{-}Gly)_n$; – – – $[Leu\text{-}Ser(OBzl)\text{-}Leu\text{-}Gly]_n$ b) ——— $(Ser\text{-}Leu\text{-}Gly)_n$; – – – $[Ser(OBzl)\text{-}Leu\text{-}Gly]_n$. Concentration 0.3 g l^{-1} in 1 mm thick cells.

respectively, suggest that the conformation of the polypeptides either benzylated or deprotected are identical and mainly based on an α-like helical structure.

DISCUSSION

As these polypeptides were studied originally to test the molecular model of Kennedy *et al.* (6), let us first discuss this conformation and recall its major principles. There should be a glycyl residue every four residues ; seryl is supposed to play a role in the ion translocation process as the hydroxyl group is located inside the helix channel. In addition to the fact that no experimental evidences for such a conformation have been found, this model cannot explain the formation of channels with the O-benzyl derivatives just for steric reasons ; it also cannot explain the wide range (1 to 100) of conductances observed with $(Leu\text{-}Ser\text{-}Leu\text{-}Gly)_n$ nor can it account for the conductances induced by the polytripeptides. Thus this model can be ruled out and, for the same reasons, any intramolecular model either of the β-helical type (4,5) or α-helical type (10) can be rejected. Other models, either intra or intermolecular where the translocation of ions occurs through a gliding dislocation of the backbone or through side chains sequences (11,12) are also not relevant as no channeled structure is concerned. We are thus led to contemplate other

molecular models. The wide range of conductances suggest assemblies of molecules either in an extended or a helical conformation. This type of model was proposed by Hanke and Boheim (13) for the peptide Alamethicin as a kind of barrel with varying number of staves ; the extended form of the molecules may change to a helical form under an applied voltage as discussed by Fringeli and Fringeli (14). Bundles of α helices (15) have also stimulated much interest in view of the general finding that membrane proteins are rich in α-helix. This latter model could account for the polypeptides described in this report and may explain the difference of behavior of the polytetrapeptides upon deprotection by considering different associations arising from intermolecular side chains interactions. Finally, there still remains a trivial explanation, i.e., poorly defined pores formed by micellisation of the membrane constituents (16).

Thus, the molecular nature of the ion channels that are observed with the four synthetic polypeptides is still obscure. However, the results allow to rule out the β-helix model of Kennedy *et al.* (6) and stimulate the study of other peptides for which the α-helical conformation would be well defined.

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