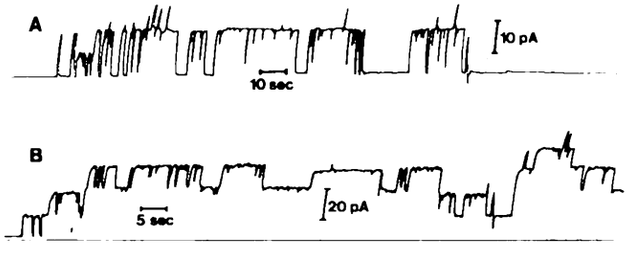
Synthetic Peptides Form Ion Channels in Artificial Lipid Bilayer Membranes

Abstract. *Ion permeability of cell membranes is considered to be mediated by ion channels. The synthesis and testing of four peptides having the sequence (Leu-Ser-Leu-Gly)$_n$ and expected to form $\beta^{12}_{6,6}$-helical ion channels in lipid bilayer membranes is reported. The two peptides capable of forming helices four turns in length have been demonstrated to form ion channels in an artificial lipid bilayer membrane system. The ion selectivity of the channels is consistent with the predicted β^{12}-helical model in which ions traversing the channel are coordinated by serine hydroxyl groups arrayed in the interior of the channel.*

Certain antibiotics such as gramicidin A, alamethicin, and amphotericin B form high-dielectric pathways through cell membranes, thereby increasing ionic permeability (*1*). These compounds have been extensively studied in artificial membrane systems as possible models of naturally occurring transmembrane ionic channels. A new class of helical conformations of polypeptide chains having the ability to form transmembrane ion channels has been described (*2*). These are regular helical structures in which individual amino acid residues have a local conformation equivalent to that in a β-pleated sheet, and they have been termed β helices. The simplest member of this class of structures, the $\beta^{6}_{3,3}$ helix (*3*), has been described (*4*) and suggested as the channel conformation of gramicidin A (*5*). The newly proposed members of the class of β helices are larger in diameter and have amino acid side chains in the interior of the helix. These new structures are particularly suited to the formation of ionic channels because ion coordination can be supplied by protein functional groups inside the helix. In general, the three-dimensional structure of a β helix is directly related to its amino acid sequence, and, for a given β helix, amino acid sequences can be chosen that should form the helix in a lipid bilayer environment. This introduces the possibility of designing artificial transmembrane channels and examining their structure-function relationships. For this reason, we have synthesized four peptides with the sequence (Leu-Ser-Leu-Gly)$_n$ and tested them for channel-forming ability in an artificial lipid bilayer system. We report here that the two peptides capable of forming helices four turns in length can form transmembrane ionic channels and that the properties of these channels are consistent with the predicted $\beta_{6,6}$-helical structure. The $\beta_{6,6}$ helix is a newly observed regular conformation of polypeptide chains and the first example of the new members of the set of β helices.

The peptides (Leu-Ser-Leu-Gly)$_n$ with n as 6, 9, and 12 (designated C-VI, C-IX, and C-XII, respectively) and the *N*-formyl derivative of C-VI, designated fC-VI, were synthesized by fragment condensation onto a Merrifield solid-phase peptide resin. The protected tetrapeptide Boc-Leu-Ser (*O*-benzyl)-Leu-Gly-OH was synthesized by both solution and solid-phase techniques according to standard methods of peptide synthesis (*6*). This peptide was then repeatedly coupled to the solid-phase tetrapeptide resin in order to generate polypeptides of the required length (*7–9*).

Completed peptides were examined for their ability to increase the conductance of planar lipid bilayer membranes. These were formed across a hole in a Teflon partition separating two Plexiglas chambers containing identical salt solutions. Membranes were usually formed from a 2 percent solution in decane of the synthetic saturated phospholipid diphytanoyl phosphatidylcho-



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Electrolyte	Lipid	Peptide	Unit Conductance (nS)
1.0 M KCl	DiPhytPC/Decane	fC-VI	1.45 ± .03
1.0 M NaCl	DiPhytPC/Decane	fC-VI	0.67 ± .06
1.0 M LiCl	DiPhytPC/Decane	fC-VI	0.43 ± .04
1.0 M NaCl	Egg PC/Decane	fC-VI	0.68 ± .03
1.0 M LiCl	GMO/Decane	fC-VI	1.70 ± .07
1.0 M NaCl	DiPhytPC/Decane	C-XII	0.65 ± .07
1.0 M LiCl	DiPhytPC/Decane	C-XII	0.45 ± .03

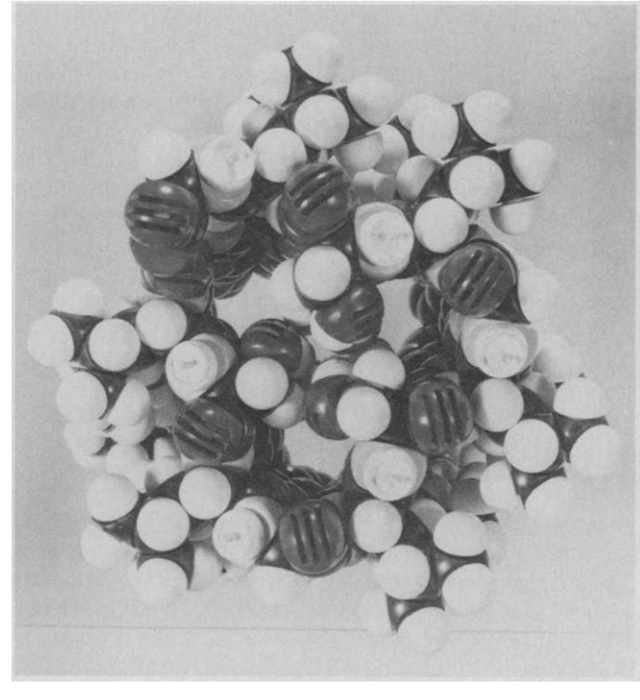

Fig. 1 (left). (A) Single channel current record obtained in the presence of fC-VI. Diphytanoyl phosphatidylcholine membrane, 1.0*M* NaCl; 20 mv. (B) Current record of a multichannel membrane obtained in the presence of fC-VI. Egg phosphatidylcholine membrane, 1.0*M* NaCl; 20 mv. In both (A) and (B), the aqueous concentration of fC-VI was ~ 20 n*M*. (C) Summary of the unit channel conductance data for the peptides fC-VI and C-XII under various conditions. Values given are means ± S.D. for at least 20 determinations. Fig. 2 (right). End view of a CPK (Corey, Pauling, Koltun) model of the $\beta^{12}_{6,6}$ helix proposed as the channel conformation of the peptides fC-VI and C-XII. The exterior of the helix is entirely hydrophobic; the interior is polar, due to the presence of serine side chain hydroxyl groups.

line (*10*). In a few experiments, egg lecithin (2 percent solution in decane) or glyceryl monooleate (0.5 percent solution in decane) was used. After the membranes were completely black, a small volume of a solution of peptide in trifluoroethanol (0.1 to 3.0 mg/ml) was added to both aqueous phases. (The final concentration of trifluoroethanol was usually 0.05 percent; concentrations 20 times greater had no effect on membrane conductance in control experiments.) A transmembrane potential of up to 100 mv was applied, and the membrane conductance was monitored. For this, membrane current was measured by an operational amplifier (Analog Devices AD41J) having a field-effect transistor, which has a very high input impedance; the amplifier, in the inverting mode, was connected to the chamber through Ag/AgCl electrodes and 3*M* KCl salt bridges.

After the addition of peptides fC-VI or C-XII to the aqueous phases at concentrations of $3.0 \times 10^{-6}M$, the membrane conductance increased by approximately three orders of magnitude. The compounds C-VI and C-IX produced no detectable increase in conductance when added to the aqueous phases at concentrations of $3.6 \times 10^{-5}M$ and $1.2 \times 10^{-5}M$, respectively. These compounds also had no effect when added directly to the membrane-forming solution at ratios of peptide to lipid as high as 0.5 (by weight). When, however, membranes were formed from solutions containing either fC-VI or C-XII at a ratio of peptide to lipid of 0.1, measured conductances were three to four orders of magnitude higher than those of membranes formed from lipid alone.

The ionic selectivity of the conductance induced by fC-VI was determined from the open circuit voltage produced by ionic concentration gradients (*11*). The cationic transference numbers for LiCl, NaCl, and KCl were found to be 0.78 ± 0.01, 0.82 ± 0.03, and 0.82 ± 0.02, respectively. These values indicate that about 20 percent of the current is carried by chloride ion. This observation is consistent with the prediction that ions traversing the channel would be coordinated by serine hydroxyl groups. The hydroxyl group is an amphivalent ligand and may coordinate either anions or cations.

In the presence of very low concentrations of fC-VI or C-XII ($\sim 10^{-9}M$), the current through the membrane was observed to fluctuate in discrete steps, indicating that both these compounds form ion channels through the membrane. The conductance of the individual channels was measured under various conditions of electrolyte and lipid composition. The results are summarized in Fig. 1, where typical single-channel current traces are also shown.

Our experimental results are consistent with the β^{12}-helical model for the channel conformations of the peptides fC-VI and C-XII, shown in Fig. 2. The peptide chain is wound into a large helix containing four turns of 12 residues each. The exterior of the helix is hydrophobic, due to the presence of leucine side chains, whereas the interior is hydrophilic, due to the radial array of serine hydroxyl groups. Ions may be coordinated by these polar side chains inside the helix. The C-XII channel is formed by a single, continuous peptide chain. The fC-VI channel is formed by two fC-VI monomers dimerized end-to-end through the *N*-formyl groups at their amino terminals.

Although the cross-sectional area of the β^{12} helix is only three to four times as large as that of the β^{6} helix, the unit conductance of fC-VI is about 50 times that of gramicidin A under similar conditions (*12*). This suggests that the serine hydroxyl groups in the fC-VI channel provide more favorable ion coordination than the peptide carbonyl groups of gramicidin A. Because both anions and cations can enter the fC-VI channel, it is likely that the channel is not restricted to single occupancy, and a higher flux density can be maintained. As has been demonstrated for gramicidin A (*13*), the unit conductance of fC-VI channels is significantly higher in glyceryl monooleate–decane than in phosphatidyl choline–decane membranes.

The fact that C-VI produced no increase in conductance implies that dimerization via the formyl groups is required for formation of the fC-VI channel. In addition, the lack of activity of the peptide C-IX indicates that three turns of β helix are of insufficient length to span the membrane interior. Because the channel formed by fC-VI or by C-XII is only about 20 Å in length, channel formation must induce localized thinning of the membrane hydrocarbon interior to reduce it from its normal value of 47 Å for phospholipid–decane films. This probably occurs by exclusion of solvent (*14*) from the area surrounding the channel.

Model compounds such as the synthetic peptides described here could prove valuable in the elucidation of the structure of naturally occurring transmembrane ionic channels. The β^{12} helix is, to our knowledge, the first regular helical structure to be described that contains amino acid side chain functional groups in the interior of the helix. Hille has suggested that naturally occurring channels contain such groups (*15*). Furthermore, while the β^{12} helix requires that every fourth amino acid residue within its sequence be either a D-amino acid or glycine, β helices exist (*2*) that have no requirement for D-residues at all. These helices may prove to be important features of natural membrane proteins and ion channels.

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3. In the notation proposed by Urry (*4*), the superscript designates the number of residues per turn, and the subscripts refer to the number of peptide bonds in each turn whose carbonyl groups point respectively parallel and antiparallel to the helical axis. Abbreviations for the amino acids mentioned are Leu, leucine; Ser, serine; Gly, glycine; also, Boc, *t*-butyloxy-carbonyl.
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7. The resin used was 1 percent cross-linked polystyrene containing 1.34 mEq chloride per gram of resin. Coupling steps employed dicyclohexyl carbodiimide, hydroxybenzotriazole, and the protected tetrapeptide, all in twofold excess, and were followed by acylation with *o*-nitrophthalic anhydride (*8*). Portions of the resin were removed at the appropriate stage, and the peptide cleaved with HBr in trifluoroacetic acid. A portion of the tetracosapeptide was formylated with *p*-nitrophenyl formate (*9*) to generate the *N*-formyl peptide fC-VI.
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