*Biochimica et Biophysica Acta*, 291 (1973) 317–320



## BBA Report

BBA 71162

# A synthetic transmembrane channel

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(Received December 5th, 1972)

SUMMARY

Based on a recently published molecular theory of ion conducting channels considering gramicidin A and related conformations, several linear polypeptides have been prepared and tested for channel formation in lipid bilayer studies. Synthetic polypeptides containing the repeating sequence (L-Ala–L-Ala–Gly) have been found to form transmembrane channels. To our knowledge this is the first report of wholly synthetic transmembrane channels which are related only by conformational arguments to known naturally occurring polypeptides.

A molecular theory of ion conducting transmembrane channels has recently been proposed which contains a mechanism for a field-dependent transition between conducting and non-conducting conformations of the same polypeptide[1]. The transition involves interconversion between two transmembrane structures with different net dipole moments along their axes. One is a closed spiral structure and the other is an open, channel-containing, helical structure. In an exemplary case four of the approximately six residues per turn in a helical configuration rotate to form hydrogen bonds of β-turns while the two remaining peptides retain their intra-turn hydrogen bonding. The theory suggests that, among other sequences, the structure *N*-formyl-(L-Ala–L-Ala–Gly)$_n$ would form such channels. As an initial step in considering the proposed mechanism, we have prepared three separate polypeptides involving the repeat sequence (L-Ala–L-Ala–Gly). The present is a report of the lipid bilayer studies on these synthetic polypeptides.

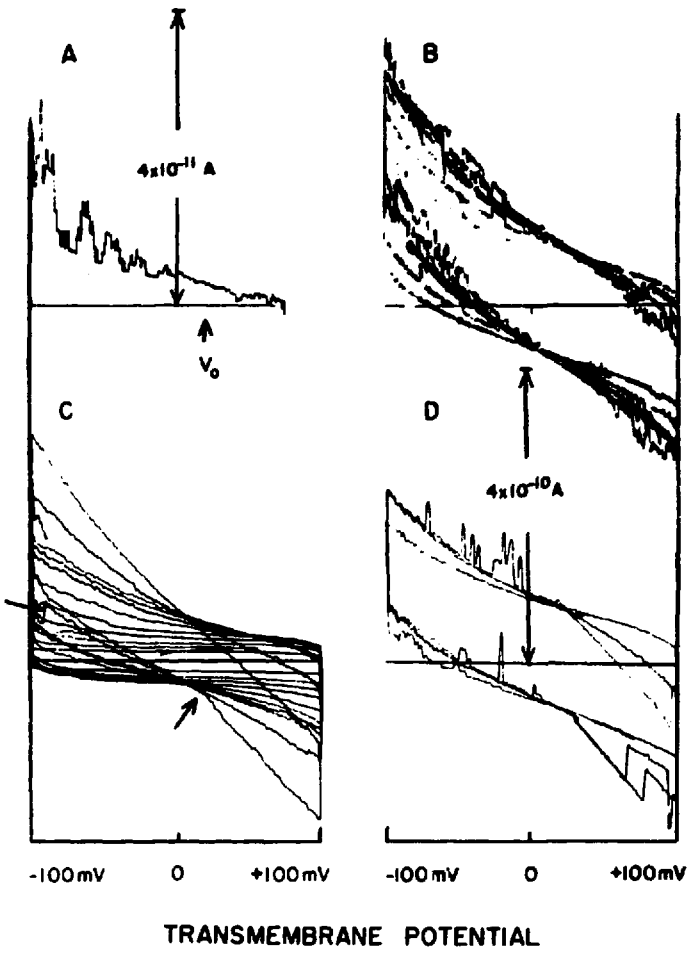


Fig.1. Actual current (vertical displacement) *vs* voltage(horizontal displacement) tracings following addition of synthetic polypeptides to the lipid bilayer system. The lipid bilayer was formed with soy bean phosphatidylcholine dissolved in *n*-decane. A. Initial appearance, on a sensitive scale, of conductance steps after addition of *N*-formyl-(L-Ala–L-Ala–Gly)$_4$OMe (Preparation II). As the voltage is swept from left to right, the frequency of conductance steps increases. The conductance increments are approximately $2.6\cdot10^{-11}$ $\Omega^{-1}$. Sweep rate, 2 mV/s. B. Continuous tracings as the voltage is swept back and forth in a triangular wave fashion following the addition of *N*-formyl-L-Ala–Gly–(L-Ala–L-Ala–Gly)$_4$OMe (Preparation III). Steps are observed as channels turn "on" and "off". As channels remain "on" the slope of the curve increases by obvious increments. Sweep rate, 2 mV/s. C. With decreased sensitivity the continuous build-up of conductance is observed as an increasing slope in the time period following addition of Preparation I. The events indicated by the arrows are the first appearances of larger conductance steps, $2.8\cdot10^{-10}$ $\Omega^{-1}$ and $1.1\cdot10^{-9}$ $\Omega^{-1}$. D. Again with decreased sensitivity, which does not allow resolution of the smaller conductance steps, Preparation I again shows the onset of large channels which "turn on" on the lower right-hand side of the tracing and "turn off" at nearly the same voltage on the upper curve. The channels only sporadically appear on the left-hand side. These are the largest channels with $\Delta g \approx 0.8\cdot10^{-9}$ $\Omega^{-1}$. Sweep rate, 20 mV/s.

The details of the preparations, including spectroscopic characterization, will appear in subsequent publication (Ohnishi, T., Johnson, B. and Urry, D.W., in preparation). Here we only briefly describe each preparation. The first effort referred to as Preparation I, was to synthesize *N*-formyl-(L-Ala–L-Ala–Gly)$_5$OMe. Due to the insolubility of *N*-formyl-(L-Ala–L-Ala–Gly)$_5$OH, it could be only partially methylated and, in addition,

solubilization for addition to the bilayer solution required dissolution in trifluoroacetic acid followed by immediate dilution with trifluoroethanol. In the resulting 5% trifluoroacetic acid solution, there is a slow deformylation. The second Preparation, II, was *N*-formyl-(L-Ala–L-Ala–Gly)$_4$OMe. In this case, the methylation was achieved in a transesterification step on removal from the resin. The product is soluble in trifluoroethanol. The third Preparation, III, was *N*-formyl-L-Ala–Gly–(L-Ala–L-Ala–Gly)$_4$OMe and the product is soluble in trifluoroethanol. The latter preparation would vary the capacity to form end-to-end hydrogen-bonded association. All peptides were prepared by the solid-phase method.

The peptides were tested for channel formation by methods previously used for the same purpose on extracts from excitable tissue[2]. The method consists of a triangular wave voltage sweep across a bilayer with 150 mequiv/l of $K^+$ on one side, and of $Na^+$ on the other side. The anions on both sides were 120 mequiv/l $Cl^-$ and the remainder phosphate buffer at pH 6.8. The current–voltage ($I-V$) curves were then recorded on an $X-Y$ plotter. As with all single channel work, extensive controls were run to establish the absence of channels and to demonstrate a stable "baseline" tracing prior to adding the peptide. For the purpose of verifying independence of lipid source, three lipids, soy bean phosphatidylcholine (Sigma type II, partially purified on neutral alumina), chromatographically pure bovine phosphatidylserine and synthetic dioleyl phosphatidylcholine, were used at a concentration of 25 mg/ml in *n*-decane. The first lipid gave bilayers with a steady state capacity of $0.46 \pm 0.01\ \mu F/cm^2$.

All three peptide preparations exhibit channel behavior with weak $K^+$ selectivity, $V(K^+/Na^+) \approx 20$ mV. As seen in Fig.1A, Preparation II, *N*-formyl-(L-Ala–L-Ala–Gly)$_4$OMe, exhibits initial conductance development in discrete increments of $\Delta g(K^+) \approx 2.6 \cdot 10^{-11}\ \Omega^{-1}$ as the voltage is swept to 100 mV. Preparation III gave channels of similar increments and selectivity, but of less overall activity (Fig.1B). Preparation I which is a mixture of four species, $H^+$(L-Ala–L-Ala–Gly)$_5$O$^-$, $H^+$(L-Ala–L-Ala–Gly)$_5$OMe, *N*-formyl-(L-Ala–L-Ala–Gly)O$^-$ and *N*-formyl-(L-Ala–L-Ala–Gly)$_5$OMe, exhibited three distinct sizes of conductance quanta, one the same size as Preparation II and the additional two larger, *i.e.* $2.6 \pm 0.2 \cdot 10^{-10}\ \Omega^{-1}$ and $1.0 \pm 0.2 \cdot 10^{-9}\ \Omega^{-1}$.

Fig.1C shows cumulative build-up of the small channels, and in places channels of larger size are resolved. Fig.1D shows the appearance of two of the largest size channels on a background of the smaller. These large channels are voltage dependent and turn on at about 30 mV (the switching potential) where the current is being carried predominantly by $Na^+$. In this respect, they are similar to $Na^+$-selective channels from excitable tissue[2], only the selectivity and the magnitude of conductance (the conductance of the synthetic channels being as much as three times greater at same electrolyte concentration) are different, possibly indicating multiple channel formation[3]. On the other hand, the specific ion-dependent turning on is similar to the action of alamethicin, now known to be channel forming[4] where, in contrast to the above, $K^+$ is the ion which gives the lower switching potential[5]. It is noteworthy that the voltage-dependent turning on and off was observed in the preparation containing charges at the ends of the polypeptides.

This work was supported by U.S. Public Health Service Grant GM-18721, by National Science Foundation Grant GB-31665, and by the Mental Health Board of Alabama. The authors are particularly indebted to Mr T. Ohnishi, research assistant in the Laboratory of Molecular Biophysics, for synthesizing the peptides and to Dr Brian Johnson for advice on the syntheses.

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