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Conference Abstract

Discreteness of Conductance Change in Black Lipid Films*

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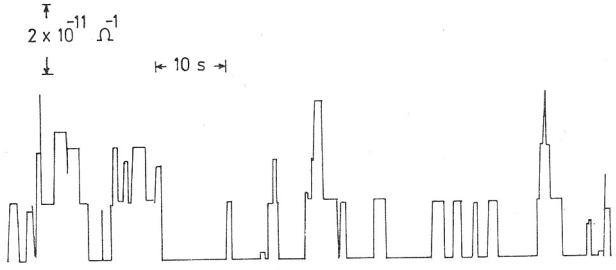
The mechanisms by which inorganic ions cross biological membranes are still effectively unknown. However, it has become very clear during the past few years that the proteins or other macromolecules in the membrane are much more likely to be involved in this process than are the lipids. Thus, experiments with artificial lipid membranes have revealed no indication of the necessary ion transfer or ion selectivity properties among the naturally occurring lipids, whereas many small macromolecules, including some polypeptides, are known to be entirely adequate in these respects.

Mechanistic studies on the active macromolecules present many difficulties. For example, the necessary ion transfer is brought about by surface concentrations of the macromolecule which are far below that which can be studied by conventional surface chemical techniques. Indeed, the conductance change in the membrane is often the only parameter which is readily measurable. Such conductances are, however, obviously difficult to interpret in terms of molecular events. On the other hand, the large conductance change per macromolecule, and the very low intrinsic conductance of the lipid film, make it feasible in certain systems to detect and examine individual or molecular conductance channels.

One such system is the polypeptide antibiotic gramicidin A (M. wt. 1883) incorporated into black lipid membranes formed from glyceryl mono-oleate (or phospholipids) and aliphatic hydrocarbons. At very low concentrations of the polypeptide the current through the membrane, under constant applied potential, fluctuates as shown in the Figure[1–3]. The step changes in the current are quite well defined and correspond to the smallest observable conductance change. The channels occur randomly and the larger fluctuations are integral multiples of the basic unit.



Conductance transitions for a membrane of glyceryl mono-oleate and hexadecane in the presence of a very small amount of gramicidin A. The electrolyte was 0.5 M NaCl, the membrane area 8×10^{-4} cm² and the temperature 23 °C.

Transference number studies show that the current is carried only by certain univalent cations e. g. hydrogen ions, all the alkali metal ions and ammonium, but not, for example, tetramethylammonium.[4] No anions appear to penetrate the channel.

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The conductance ratios for the various ions in the unit channel follow quite closely the single ion conductance ratios at infinite dilution in water, and the activation energies for the diffusion of the ions in the channel are also similar to those for diffusion in water. The standard free energies for the transfer of the ions from the aqueous phase to the channel are small (*ca.* —2 kcal mole^{-1}). These data suggest that the channels formed by the polypeptide contain sufficient water for the hydrogen ion to retain its anomalous diffusion mechanism and for all the ions to pass through without substantial dehydration.

It is also found that the conductance of the molecular complex is, within the accessible range, independent of the thickness of the membrane. This observation, together with those discussed above, is consistent with the idea that the gramicidin forms a rigid pore-like structure in the membrane. In a recent paper, Urry *et al.*[5] have proposed that this structure consists of the polypeptide coiled into a left-handed helix of sufficient amino acids per turn to leave a tunnel along its axis wide enough to permit the passage of water and certain ions. Such a structure is not yet established beyond doubt, but is attractive in that it leaves all of the many non-polar side chains in gramicidin on the outside of the helix to interact with the lipid and only the polar peptide linkages lining the pore. The individual helical molecules could join head to head or tail to tail by means of hydrogen bonds and there is some evidence in membranes of 'biological' thickness (25—50 Å) that the operative conducting complex is a dimer.

The precise mechanism by which the channels open and close remains obscure. It has, however, been shown that, in general, there is an equilibrium in the membrane between conducting and non-conducting gramicidin complexes, and that thinner membranes tend to favour the conducting state.

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