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Rapid report

Valinomycin acts as a channel in ultrathin lipid membranes

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Abstract

When the thickness of monolayer membranes formed by bolaform archaeal lipids is reduced to the approximate length of two valinomycin molecules, the zero-current conductance does not show any more a linear dependence on valinomycin concentration; instead, a quadratic behaviour is observed. This suggests that a dimer permeation pore is formed and therefore the conduction mechanism changes from carrier to channel.

Keywords: Valinomycin; Ultrathin lipid membrane; Bolaform archaeal lipid; Conduction channel; Permeation

The original task of employing lipid bilayers to mimic biological processes by embedding polypeptides or proteins [1–7] has been extended in an effort to produce materials with nanoscopic dimensions. Classical black lipid membranes (BLMs) can be easily transformed into self-assembled lipid bilayers on solid substrates [8] or polymer supported bilayers [9–12]. Potential technological applications are in areas such as device technology, drug delivery, biosensors and the simulation of cell surfaces [8,13–15]. The relevance that model systems can assume in nanoscopic applications induced us to reconsider the clear cut division that has been set between two transport mechanisms, namely carriers and channels [1–4]. The first type of transport involves molecules which are embedded in the membrane and shuttle specific ions from one side to another. The other consists of ionic channels formed by molecules which during the transport are fixed with respect to the hydrophobic core of the membrane. Here we show that when the membrane thickness is reduced to the approximate length of two valinomycin molecules, the conduction mechanism changes from carrier behaviour to channel behaviour, due to the formation of a dimer permeation pore.

The membranes were formed with a new procedure [16] in which, unlike the Montal and Mueller technique [17], the monolayer is formed only on one side of the teflon partition. This was made possible by the lipid used (glycerol-dialkyl-nonitol tetraether, GDNT) which has two polar heads [18,19], as shown in Fig. 1. When this lipid, dissolved in an organic solvent, is spread on an aqueous surface at the air–water interface it enters a metastable state [20]. In this state the long axis of the molecule is oriented perpendicular to the interface. The monolayer is transferred on the teflon hole by simultaneously raising the two water levels (Fig. 2a,b). The molecular structure of these membranes has been investigated by a series of

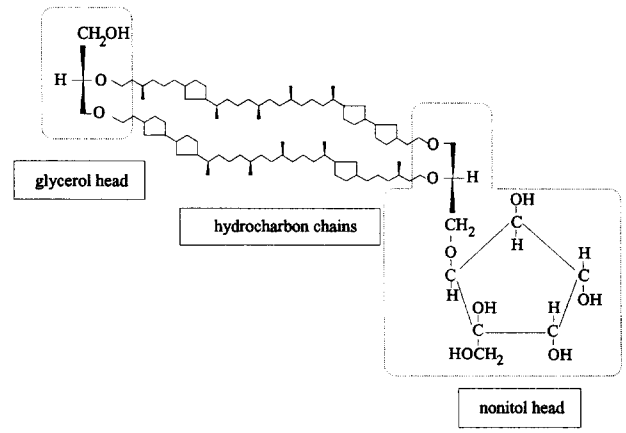

Fig. 1. Chemical structure of the bipolar lipid GDNT. The structure of the nonitol head is reported in accord with Ref. [27].

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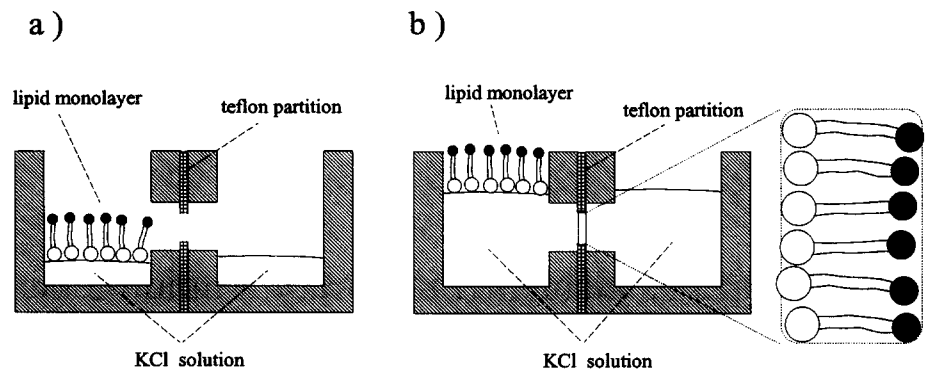

Fig. 2. (a and b) A schematic drawing (not to scale) of the modified Montal and Mueller technique which allows the formation of monolayer membranes. The experimental details can be found in Ref. [16].

experiments such as conductance in the presence of valinomycin, voltage-dependent capacitance and electroporation [16]. The results indicate that the membrane is asymmetric and that it is formed by a single monolayer (Fig. 2b). Its hydrophobic thickness, d, is 30 ± 4 Å, when the lipid is dispersed in a volatile solvent, like chloroform. Thinner membranes ($d = 24 \pm 2$ Å) can be formed by dispersing the lipid in n-decane. Details on the structure of these membranes can be found in Ref. [21]. As far as we know, these are the thinnest membranes ever formed by lipid molecules.

This work raises the question whether a shuttle carrier mechanism can be active in these unusually thin membranes. To elucidate this point, the membrane conductance as a function of valinomycin concentration has been measured. According to the carrier theory [1,22,23], the membrane conductance is a linear function of the carrier concentration. This behaviour has been experimentally observed for most of the membranes previously analyzed. On the other hand, in some cases, a non-linear behaviour in the conductance–concentration curves with a slope greater than one had been observed for valinomycin and derivatives [24]. However, no correlation had been shown between membrane thickness and the conductance behaviour.

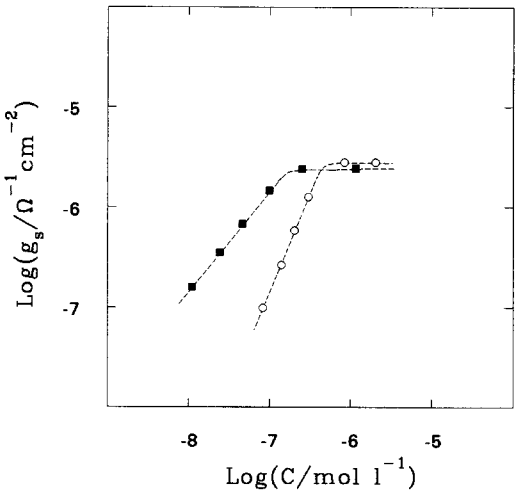

Fig. 3. Typical behaviour of membrane conductance, g_s, as a function of valinomycin concentration, C, in a log–log plot. Measurements have been repeated over ten different membranes. Error bars lie whithin the plotted symbols. (■) GDNT/chloroform membranes. The slope of the straight line is 1.1 ± 0.1. (○) GDNT/decane membranes. The slope of the straight line is 1.9 ± 0.1.

Fig. 3 compares the results obtained on GDNT/chloroform and GDNT/decane membranes. A linear behaviour is displayed by GDNT/chloroform membranes. In contrast, a quadratic behaviour of conductance vs. concentration is detected in the ultrathin GDNT/decane membranes. This is a very intriguing result, since it suggests that two carrier molecules are necessary to mediate ionic permeation. The valinomycin molecules, whose conformation is roughly a 12 Å thick bracelet [25] should therefore form a dimer which, in turn, constitutes a permeation pattern. The details of this transport mechanism are not trivial, since the thickness of two aligned molecules is slightly lower than the total membrane thickness which, including the polar heads, can be estimated to be around 30 Å. It is possible that the dimer pair is able to induce a membrane deformation, as in the case of gramicidin [26]. Moreover, on the analogy of gramicidin transport, it should be possible to detect single channel conductance.

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