Short communication

Increase of Ionic Conductance of Amphotericin B Channels under Antibody Action

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According to recent studies, monoclonal antibodies, due to their high specificity, can be used as a unique instrument ot study the properties of ion channels in cellular membranes. However such studies are difficult because the mechanisms of antibody-channel interaction as well as the structure of cell membrane channels are not sufficiently well understood. The present study concerns the mechanism of the channel-antibody interaction. For this purpose a channel of known structure (formed of polyene antibiotic amphotericin B and cholesterol in a bimolecular lipid membrane by adding the antibiotic at one (*cis*) side of the membrane) was investigated.

According to current theory, adding the amphotericin B to one side of a lipid bilayer induces the formation of asymmetrical "semipores" in the *cis*-monolayer of the membrane. A "semipore" can pierce the membrane for a short time to form an ion channel. If amphotericin B is added to both sides of the lipid bilayer, the amphotericin B and cholesterol molecules form symmetric ion channels which consist of two asymmetric "semipores" occurring in the opposite monolayers of the bilayer and joined to each other by hydrogen bonds (De Kruyff and Demel 1974; Ermishkin et al. 1977; Brutian 1982).

In order to elucidate the possibility of obtaining antibodies against the amphotericin channel, rabbits were immunized with amphotericin B (1 mg) suspension in physiological solution (0.5 ml) mixed with complete Freund's adjuvant (0.5 ml). Rabbits were injected with this suspension three times intraperitoneally and once into the lymphatic nodes. The interval between injections was two weeks. One week after the last injection 50 ml of blood was taken from each rabbit. Immunoglobulins were isolated from the blood serum by sedimentation with ammonium sulphate (Kabat and Mayer 1964).

Planar lipid membranes were assembled by Montal-Mueller's technique of two lipid monolayers (Montal and Mueller 1972). Total bovine brain lipids were used for this purpose. The membrane diameter was 100 μm. The asymmetrical channels of amphotericin B and cholesterol were formed in the membrane by adding the antibiotic to the aqueous solution at *cis*-side of the bilayer.

Experiments on the lipid membrane revealed that in the preparation obtained there were antibodies capable of changing the properties of an asymmetric amphotericin channels. It should be emphasised that the antigenic determinants there antibodies bind to are situated on the channel at the *trans*-side of the membrane only. The binding of antibodies to determinants serve to greatly increase the probability of channel opening without influencing the amplitude of this channel in the membrane.

In order to determine the class of immunoglobulins and to estimate the stoichiometric ratio of the channel-antibody complex, monoclonal antibodies were used.

Mouse hybridomas and monoclonal antibodies were obtained by a conventional technique (Eshhar 1985). Monoclonal antibodies were isolated from the cell supernatants by sedimentation with ammonium sulphate (Kabat and Mayer 1964). The clones were selected with respect to the ability of antibodies isolated from cell supernatants to change membrane conductance in the presence of amphotericin B in the solution at the *cis*-side of the membrane. In doing so, three monoclones were found whose antibodies differed from each other only in the concentration required to produce equal effects on integral membrane conductance. In this paper the concentrations for one of these monoclonal antibodies are given.

In order to characterize monoclonal antibodies the antibody-linked immunosorbent assay for class-specific antibody activity was conducted (Eshhar 1985). Class-specific antibodies from "Sigma" chemical company (St. Louis, MO. USA) were used for this purpose. The immunoassay showed that the selected monoclonal antibodies belonged to the IgM class. Structure and molecular weight of immunoglobulins of this class are well characterised (Glynn and Steward 1981).

The effect of monoclonal antibodies on single amphotericin channels was analogous to that of polyclonal antibodies isolated from rabbits immunized with amphotericin B. Fig. 1*a* shows jump-like changes in transmembrane current in the presence of a low concentration of amphotericin B at the *cis*-side of the symmetric membrane. Record in Fig. 1*b* shows the intermediary process after antibody addition. Approximately 30 min following the addition of monoclonal antibodies to the *trans*-compartment of the cell the transients ceased and current jumps appeared which are presented in Fig. 1*c*. The kinetics and amplitude of these jumps did not alter throughout the observation period (10 min). The amplitude of such jumps was equivalent to those exhibited for the amphotericin channel in the absence of antibodies. Nevertheless, the probability of channel-opening for this channel was much greater than that for the amphotericin channel. It is quite possible that the increased current noise in Fig. 1*c* compared to that for the open-state current of channel without antibodies

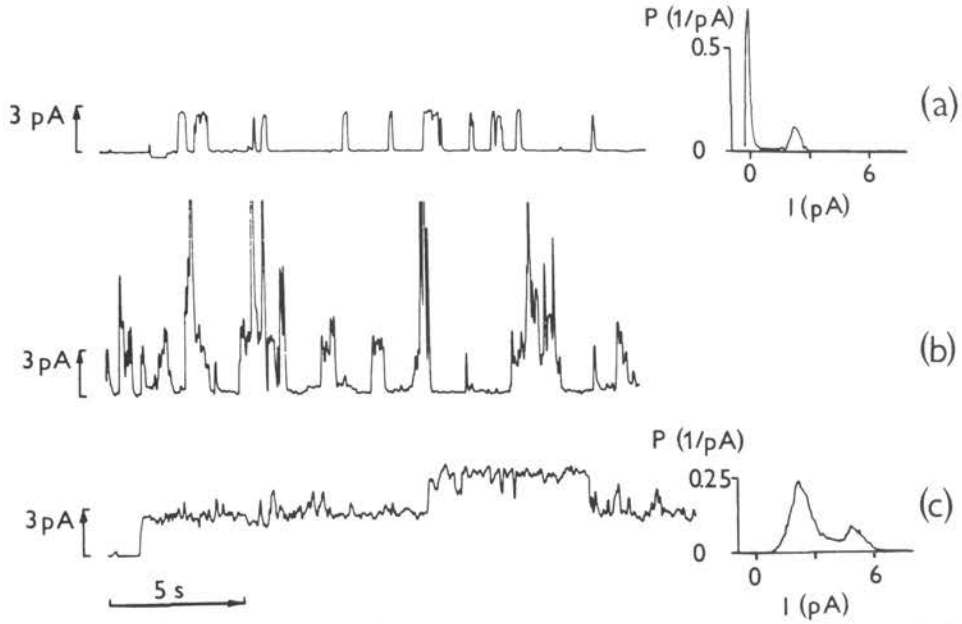

Fig. 1. Effect of antibodies on a single amphotericin B channel in a membrane. (*a*), record of transmembrane current jumps in the presence of 10 nmol/l amphotericin B in the *cis*-compartment and in the absence of antibodies in the cell. (*b*) and (*c*), records of transmembrane current jumps 15 and 30 min, respectively, after adding monoclonal antibodies (1 mg/l) to the *trans*-compartment. The right sections illustrate the probability density function of current amplitudes during single channel events. The membrane voltage was 325 mV, the positive sign was in the *cis*-compartment. The electrolyte concentration in the cell was 2 mol/l KCl.

(Fig. 1*a*) is a consequence of rapid channel switchings which are not registered because of the high time constant of the recording system (10 ms).

Fig. 2 shows the manner in which the integral conductance of the membrane increases in the presence of 1 μmol/l amphotercin B in the *cis*-compartment after addition of monoclonal antibodies to the *trans*-compartment up to a concentration of 0.7 mg/l. Measurements at lower amphotericin B concentrations and higher antibody concentrations $[A]$ revealed that in the initial period of time (t) after adding antibodies the integral conductance of membrane is well approximated by the function: $G \sim [Ch]^{\alpha} . [A]^{\beta} . t^{\gamma}$ where $[Ch]$ is the concentration of amphotericin channels in the membrane before antibody addition, $\alpha = 1 \pm 0.2$, $\beta = 3 \pm 0.6$, $\gamma = 3 \pm 0.6$.

After reaching high conductance values, the antibodies were removed from the solution. But the high conductance persisted throughout the observation time (15 min), which points to a tight binding of antibodies to the channel.

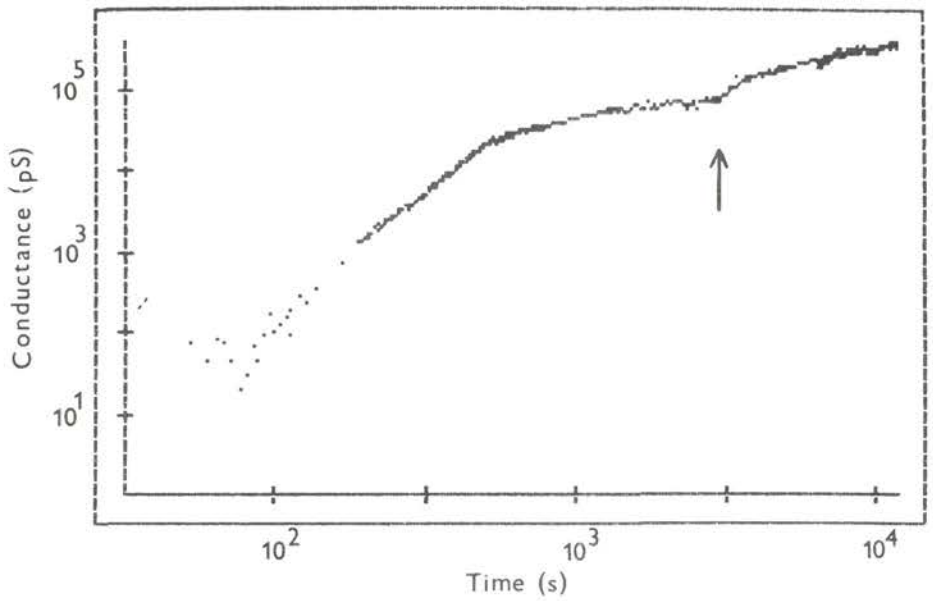

Fig. 2. The increase in the conductance of a lipid membrane with time in the presence of 1 μmol/l amphotericin B in the *cis*-compartment after adding monoclonal antibodies (0.7 mg/l) to the *trans*-compartment of the cell. The time is zero at the moment of antibody addition. The arrow shows the time moment of additional introduction of the same quantity of antibodies to the *trans*-compartment. The membrane voltage was 25 mV, the positive sign was in the *cis*-compartment. The electrolyte concentration in the cell was 0.1 mol/l KCl.

The antibodies showed a high specificity. For example, antibodies obtained at a concentration of 5 mg/l had no effect on the channels in which amphotericin B and cholesterol were replaced by their analogs levorin and 5α-androstan-3β-one. Polyclonal immunoglobulins isolated from the serum of the control nonimmunized mouse at a concentration of 5 mg/l did not change the properties of the amphotericin B channels.

Monoclonal and polyclonal antibodies did not change the properties of the asymmetric channel when added to the *cis*-side of the membrane at a concentration of 2 g/l. The obtained antibodies did not change the properties of the symmetric channels formed by adding amphotericin B at both membrane sides. In these experiments the concentration of antibody molecules in the cell was much higher then that of amphotericin B molecules.

In relation to the mean stoichiometric ratio of the channel-antibody complexes the following points are of interest. The amphotericin channel formed by eight amphotericin B molecules and by eight cholesterol molecules has a rota-

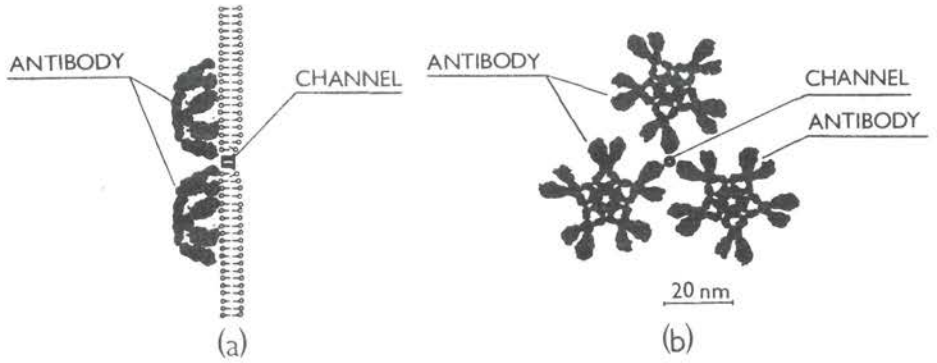

Fig. 3. Schematic representation of an amphotericin B channel and three antibody molecules bound to it. On the left, only two antibody molecules bound to the channel are shown for clarity.

tional 8-fold symmetry (De Kruyff and Demel 1974). Hence it may be assumed that the channel has a valency of 8 to react with the antibody. The channel size and antibody size are known (De Kruyff and Demel 1974; Glynn and Steward 1981). Therefore we can schematically represent a channel with three antibody molecules bound to it at the *trans*-side of the membrane as shown in Fig. 3. The antibodies are shown so as they do not close the entrance to the channel, since on their binding with the channel the amplitude of channel and its selectivity for K^+ and Cl^- change only slightly. It follows from the Fig. 3 that for steric reasons no more than three antibody molecules may bind to one channel.

Antibodies of class IgM when binding to a given antigen can have a valency from one to ten (Glynn and Steward 1981). Stoichiometry for the complex of channels with antibodies (due to their polyvalency) can change at varying concentration ratios. Two extremes may be distinguished: 1) the antibody concentration is much greater than antigen concentration; 2) antibody concentration is much lower than antigen concentration. In the first case complexes must be formed consisting of one channel with the maximum number of antibodies bound. In the second case the complexes will consist of an antibody molecule bound to a maximum number of channels (Friemel and Brock 1983).

In a case whereby membrane conductance is determined by a complex consisting of m channels and n antibody molecules, theoretical analysis indicates that the integral conductance of the membrane with incorporated channels in the initial period after addition of antibodies must increase as follows: $G \sim [Ch]^m . [A]^n . t^{m+n-1}$. According to the experimental data, conductance increases linearly with increasing $[Ch]$ and as the function of the third degree of $[A]$ and t upon increasing antibody concentration and time. Therefore it may be

assumed that for [*Ch*] and [*A*] used the complex responsible for membrane conductance consists of one channel to which three antibody molecules are bound. The formation of such structures (Fig. 3) seems to increase strongly the probability for the channel to be open (Fig. 1*c*). We can assume that trace in Fig. 1*b* reflects the intermediary structures appearing under formation of the complex presented in Fig. 3.

Thus, the antibody concentration used [*A*] is much higher than that of channels [*Ch*], so that finally complexes essentially are formed consisting of one channel with the maximum number of antibodies bound to its antigenic determinants. It may be assumed that three antibody molecules shown in Fig. 3 can form, due to mutual attraction, an integral structure presenting a "semipore" firmly bound to the "semipore" formed by amphotericin B and cholesterol in the membrane.

Thus, monoclonal antibodies (class IgM) against an asymmetric channel formed in a lipid bilayer by polyene antibiotic amphotericin B and cholesterol were obtained for the first time. It was shown that in this channel the antigenic determinants of binding, by means of which the antibodies change the channel properties, are situated at the *trans*-side of the membrane only. Three antibody molecules bind to the channel thus strongly increasing the lifetime of the channel in the open state.

The obtained evidence is of interest for elucidating the general features of interaction of antibodies with ionic channels of cellular and model membranes.

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