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RESISTANCE OF PLANAR BILAYERS OF GANGLIOSIDES

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SUMMARY

The value of electrical resistance of planar bilayers (black membranes) of gangliosides was found to be sensitive to pH, the composition of ganglioside as well as the presence of phospholipids in bilayers. The pH-dependence of resistance of black mambranes prepared from porcine brain gangliosides was determined in the presence of K^+,Na^+,and Ca^{+2}. In the absence of added phospholipids gangliosides obtained from brain of a patient with syndrom of Tay-Sachs disease are not capable to form stable black membranes, although in the presence of phospholipids the membranes are formed. The pH-dependences of resistance of black membranes obtained from mixture of phospholipids with gangliosides of porcine or human brain are compared.

INTRODUCTION

Gangliosides are amphipatic components of the extracellular matrix as well as intracellular membranes. These compounds play a critical role in such important processes as intraneuronal recognition, ion transport through membranes, synaptic transmission, reception of hormones and neurotransmitters /I-7/. At neurolipidosis (gangliosidosis), e.g. Tay-Sachs disease, the change in the relationship of the certain individual gangliosides of brain leads to a disorder of intraneuronal recognition /8-9/. However, at present

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little is known on changes in membranes caused by the pathological set of gangliosides.

In this connection it was of interest to compare properties of artificial membranes prepared from gangliosides of normal brain and from that obtained from a patient with neurolipidosis. The data on the properties of membranes formed from gangliosides are also limited. Recently Deleer at al./I0/ reported about changes in electroconductivity of the planar bilayers (black lipid membranes,BLM) formed from monoglicerides after the introduction gangliosides there.

In this report we study the electrical resistance(conductivity) of BLM formed from porcine gangliosides. Besides the resistance of membranes composed from mixtures of phospholipids with porcine or human gangliosides were determined.

MATERIALS AND METHODS

Gangliosides from porcine brain cortex and human brain cortex of a patient with Tay-Sachs disease were isolated according to Svennerhölm /I0/. Human brain of the patient(I4 years)with clinical diagnosis of Tay-Sachs disease was obtained from Bechterev's Psychoneurological Institute(Leningrad).Analytical thinlayer chromatography of gangliosides was performed by the method of Holm and Svennerhölm/I2/. The chromatographic data indicate that gangliosides from the patient have the following content of individual gangliosides: G_Q-9.8%, G_{TI}-23.7%, G_{D2}-5.9%, G_{DIa}-I3.6%,G_{D3}-5.I%, G_{MI}-I2.I%,G_{M2}-5.8%,G_{M3}-3.3%. Porcine gangliosides consist of G_{TI}-I6.3%,G_{DIb}-I8.I%, G_{DIa}-37.4%, G_{MI}-2I.7%, G_{M2}-0.9%.(Gangliosides are designated according to Svennerhölm's nomenclature/I3/).

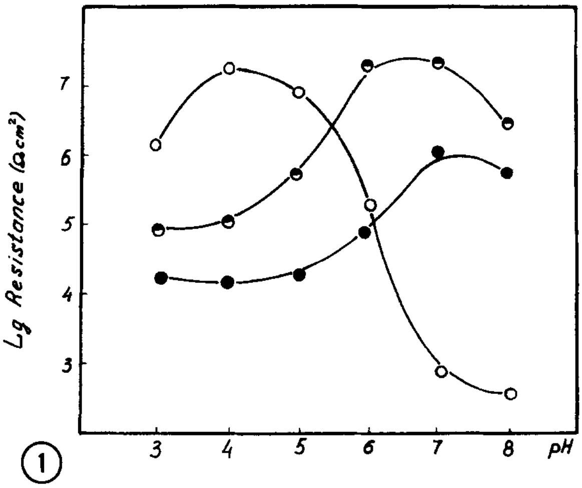
Determination of N-acetylneuramic acid in gangliosides were carried out by methods of Warren /4/ and Svennerhölm/I5/, the latter being used in the modification of Miettinen and Takki-Luukkainen/I6/.

The fraction of general phospholipids of porcine brain was isolated according to Kates/I7/.

The following methods were used for the preparation of BLM. In one of them I0 mg of gangliosides and 2 mg of cholesterol were dissolved in 0.I ml of heptane. In the second method IO mg of gangliosides and 30 mg of phospholipids were dissolved in 0.5 ml of chloroform: methanol: heptane (2:I:3). In the third method IO mg of gangliosides, 40 mg of phospholipids and 2mg of cholesterol were dissolved in 0.4 ml of chloroform: methanol: heptane (2:I:3). The mixtures obtained were vigorously shaked for several minutes. BLM was formed from these mixtures by the brush technique /I8/ across a hole (diameter 2 mm) in a teflon partition separating two compartments (V=IO ml). The compartments were filled with 0.02 M sodium acetate buffers for the measurements at pH 3.0-6.0 or with 0.02 M tris buffer for the measurements at pH 6.0-9.0. To these compartments 0.I M KCl, NaCl or $CaCl_2$ had been added. Membrane resistance was measured in a current clamp circuit using Ag-AgCl electrodes. The compartments were thermostated at 25°C. The formation of BLM checked by an optical microscope was observed during I5-20 min. after the addition of mixtures on the hole. Completely reproducible results of BLM resistance were observed at least during 5-IO min. after the formation of BLM. Standard deviation for electrical resistance of membranes prepared from the same material does not exceed 30%. Data obtained are plotted in logarithmic scale.

RESULTS AND DISCUSSION

It was found that BLM from porcine brain gangliosides is readily formed using the three above mentioned methods. The value of resistance appears to depend on pH and cations containing in the compartment of the cell. In Fig.I the dependences of resistance on pH are shown. These data were obtained for BLM, prepared from porcine gangliosides according to the first method. As it can be seen, the dependence in the presence of Ca^{+2} differs markedly from that of K^+ or Na^+. Thus, in the presence of Ca^{+2} the drop of the resistance was observed at pH above 4.0. No hysteresis phenomenon was noted at least in the region of pH 3.0-8.0 indicating the complete reversibility of the change of BLM resistance. Current-voltage plots are found to be linear in the presence of K^+ or Na^+ for basic and acidic regions (Fig.2).



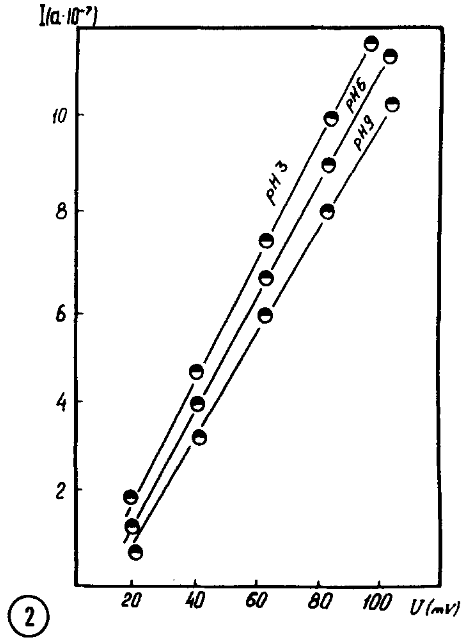


Fig. I The pH-dependence of resistance of BLM from porcine gangliosides in the presence of Ca^{+2}(○), Na^{+}(●) and K^{+}(◓).

Fig. 2 Current-voltage plots obtained for BLM formed from porcine gangliosides at different pH.

We were, however, unable to obtain stable BLM from gangliosides of a patient with Tay-Sachs disease using first or second methods. The stable planar bilayers from pathological set of gangliosides were formed only by the third method. In Fig.3 resistances of BLM prepared from porcine and pathological human gangliosides by the third method are compared. As follows, pH-dependences for BLM of porcine and human gangliosides are quite different. In the presence of K^{+} resistance of BLM prepared from porcine gangliosides does not practically depend on pH. Besides, resistance of BLM formed from pathological set of gangliosides, also does not depend on pH in the presence of Ca^{+2}. On the other hand, resistance of the

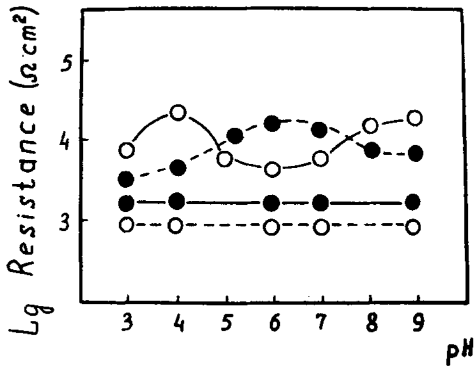


Fig.3 The pH-dependence of BLM formed according to the third method from porcine (———) and human pathological(- - - -) gangliosides (data obtained in the presence of K^+(○) and Ca^{+2}(●).

same BLM in the presence of K^+ is markedly changed at different pH. The results,presented in Fig.I and Fig.3 leads to the conclusion that the incorporation of phospholipids in ganglioside BLM brings about the change of its pH-dependence. In the presence of phospholipids the change in the value of resistance of BLM was also observed.

It is known from the titration data /I9/ that sialic groups of gangliosides are capable to bind cations, Ca^{+2} being more effective than K^+ or Na^+. In accordance with these data the results of Fig.I mean that the drop of BLM -resistance at pH above 4.0 is connected with the binding of Ca^{+2} to porcine gangliosides. The binding of K^+ and Na^+ is effective at pH above 7.0. The binding of cations of BLM, formed from porcine gangliosides, is completely reversible.

As it follows from experiments described, the binding of cations to gangliosides is sensitive, except pH, to the set of gangliosides and to the composition of BLM. The set of pathological gangliosides, does not capable to form stable BLM without the addition of phospholipids. On the other hand, the set of porcine gangliosides forms BLM both in the presence and in the absence of phospholipids. These facts raise the question on roles of individual gangliosides in the BLM - formation.

Possibly, oligasaccharide part of gangliosides plays a certain role in the formation of BLM. Thus, in membranes, prepared from gangliosides molecules of glycolipids interact not only by their hydrophobic (ceramide), but also by hydrophilic (oligosaccharide) parts. In other words, the structures of BLM obtained from porcine and human pathological gangliosides are different. It should be expected that the forms of BLM, obtained in the presence of phospholipids and without them also would be different. This follows particularly from Fig.3 as well as from the comparison of the values of resistances of corresponding BLM. It may be suggested that BLM formed from the mixture of gangliosides and phospholipids consists of interacting monolayers of these molecules or has a "mosaic" structure in which ganglioside and phospholipid bilayers are alternated. However further investigations with ganglioside mixtures from other sources and individual gangliosides are necessary to determine the BLM structure. It would be of interest also to study the properties of ganglioside bilayers containing membrane proteins.

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