Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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Version of record first published: 24 Sep 2006

To cite this article: Kazutoshi Kobayashi, Mamoru Hatakeyama, Yoshiyuki Toshima & Yasushi Tamada (2001): Response by Bylayer Membranes Containing the Labial Palpus of Fly to Stimulative Substances, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 370:1, 347-352

To link to this article: http://dx.doi.org/10.1080/10587250108030103

Mol. Cryst. and Liq. Cryst., 2001, Vol. 370, pp. 347-352

Response by Bylayer Membranes Containing the Labial Palpus of Fly to Stimulative Substances

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A group of molecules extracted from the labial palpus of fly was reconstituted into plannar bilayer membranes and liposomes to construct the taste sensors. The existence of sodium ion channel in the extract of the labial palpus of fly was recognized by the plannar bilayer membrane experiment. Fluorescent dye, Di-8-ANEPPS, which is very sensitive to the change to the membrane potential, was used to clarify the availability of this system for sensor applications. The fluorescence intensity of liposomes was proportional to the concentration of salt solution (membrane potential) added as stimulative substances. Addition of tetrodotoxin (sodium channel blocker) revealed that sodium ion channel in the extract of the labial palpus of fly was successfully reconstituted into liposome.

Keywords plannar bilayer membrane; fluorescence spectroscopy; labial palpus; membrane potential; liposome; fly

INTRODUCTION

Insects have excellent functions for their life supports. One example is a chemical communication system by the use of pheromone. Moreover, taste sensation function is essential for the purposes of not only taking

nutrient but also precluding toxic substances. The mechanism for taste sensation of fly among insects has been well investigated in an electrophysiological way.[1] The information of taste sensed by the taste organs is transferred to brain without the synaptic connection. Therefore, mimic and utilization of this insect sensing function would be an appropriate model system for the construction of biosensor. As the first step for constructing a novel biosensor system, the extract of the labial palpus of fly was reconstituted into bilayer membranes such as plannar bilayer membranes and liposomes without purification of the extract to measure the multiplied tastes (such as sourness, saltiness, bitterness, sweetness, and deliciousness). In this paper, we report on the response of these bilayer membranes to the stimuli of sodium chloride as the preliminary study.

EXPERIMENTAL

The labial palpus of fly was homogenized in 100 mM phosphate buffer, pH 7.4, containing 2 wt-% n-octyl-β-D-glucocide for extracting a group of molecules of taste organs. Plannar bilayer membrane was formed with the method reported by M. Montal.[2] Azolectin (phospholipid from soybean) as lipids and buffer (10 mM MOPS, 100 mM KCl, 0.1 mM EDTA, pH 7.4) solution as electrolyte were used. The extract of the labial palpus of fly was introduced after the formation of stable bilayer membrane. The current flow through the membrane was measured under the certain voltage. Liposome was formed with removing the surfactant, cholic acid, by dialysis for five days. The same azolectin was used as lipids. The extract of the labial palpus of fly and the fluorescent dye, di-8-ANEPPS, were incorporated into liposomes. Light scattering

and transmission electron microscopy were used to detect the size and its distribution of liposome. The typical size of liposome was 200 - 300 nm in average. The fluorescence intensity change was measured when the predetermined concentration of sodium chloride solution was injected to liposome solution in the cuvette.[3] Tetrodotoxin (TTX), which is well known as a sodium channel blocker, was used to reveal the existence of sodium channel in the extract.

RESULTS AND DISCUSSION

Figure 1 shows one example of the current traces of the plannar bilayer membranes. After the formation of the stable bilayer membrane, we introduced the extract of the labial palpus of fly. We sometimes could observe the response shown in the magnified figure. The signals shown here are similar to those of ion channels. Therefore, the existence of ion channels in the extract of the labial palpus of fly is strongly suggested.

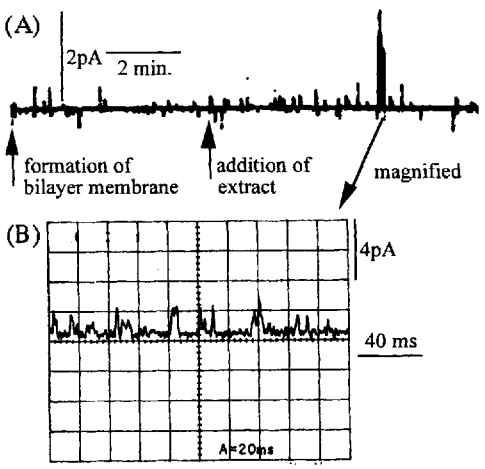

FIGURE 1 Example of current traces of the plannar bilayer membranes. (A) Trace of membrane when +30 mVof voltage was applied. (B) One part of the response (A) of membrane was magnified.

Di-8-ANEPPS had a maximum absorption at 495 nm in

methanol, and excitation wavelength was shifted to 465 nm when incorporated into liposomes (575 nm for fluorescence). We found that 520 nm of excitation wavelength was best for the detection of the membrane potential change in our liposome system. Figure 2 shows typical responses of fluorescence measurements of liposomes containing the extract of the labial palpus when sodium chloride solutions were injected. The fluorescence intensities were drastically increased to the certain values and then relaxed to the stable values depending on the salt concentration. These final values are averaged over last 20 s and summarized in Table 1, together with the data of control experiment of liposomes having no extract of the labial palpus of fly. Both the cases show straight relationship against the injected concentrations (i.e., membrane

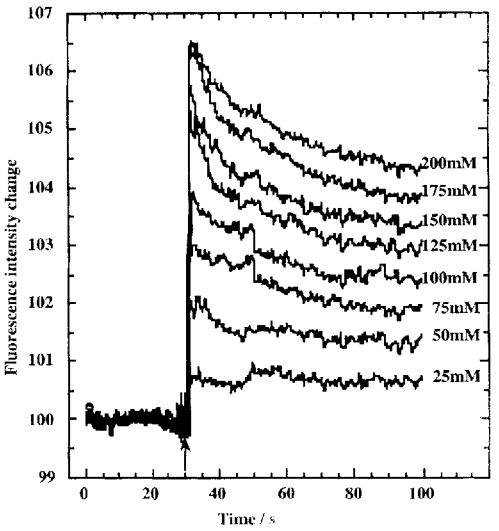

FIGURE 2 Kinetics measurements of the fluorescence intensity of the dye response in liposome containing the labial palpus of fly when various concentration of sodium chloride solutions were added as stimulative substances. The arrow shows the injection point of the salt solution. Excitation and emission wavelengths were 520 and 575 nm, respectively.

TABLE 1. Relative fluorescence intensity changes of the dye response in liposomes when various concentration of sodium chloride solution were added as stimulative substances. The data of control experiment (without the extract of the labial palpus of fly) was also shown. The intensity before the injection of sodium chloride solution was normalized to 100.

NaCl concentration injected / mM	Liposomes with extract	Liposomes without extract
25	0.648	0.825
50	1.35	1.61
75	1.90	2.23
100	2.46	2.70
125	3.00	3.27
150	3.44	3.64
175	3.93	4.18
200	4.46	4.78

TABLE 2. Relative fluorescence intensity changes of the dye response in liposomes when various concentration of sodium chloride solution were added as stimulative substances. The data of control experiment (without the extract of the labial palpus of fly) was also shown. The intensity before the injection of sodium chloride solution was normalized to 100. TTX was added to block sodium ion channels.

NaCl concentration injected / mM	Liposomes with extract	Liposomes without extract
50	101.63	101.61
100	102.77	102.70
150	103.68	103.75
200	104.64	104.71

potential). There is a clear difference between these data. The values obtained from liposomes containing the extract were smaller than those of control experiment in some degrees. This is probably because sodium ion movement through the ion channels in liposomes would cancel out the membrane potential to some extent. TTX was employed to prove

this hypothesis and the data are shown in Table 2. Fluorescence responses were settled regardless of the existence of the extract of the labial palpus of fly. Therefore, we can conclude that sodium ion channels in the extract of the labial palpus of fly were successfully reconstituted into liposomes. Due to the results above, we believe we are able to apply this method for the detection for sensing sugars and amino acids.

Acknowledgements

We would like to thank Prof. I. Shimada and Dr. M. Koganezawa (Tohoku Univ.) for providing us fly larvae and also for helpful discussion. We also thank Dr. N. Ishii (NIBH) for observing TEM pictures and Mrs. H. Saito for measuring fluorescence spectroscopy.

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