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Molecular recognition in a reconstituted tumor cell membrane

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Abstract

The design of an immunoliposome system for molecular recognition using reconstituted, hydrogel-supported bilayer lipid membranes (sb-BLMs) is described. By monitoring the electrical properties, two kinds of recognition are feasible: (i) the human bladder tumor cells, Ej and its antibody BDI-1, the lifetime of the reconstituted membrane is 42 min; and (ii) the human rectum tumor cells, LOVO, the life of the reconstructed membrane is more than 40 min, the same as conventional BLM. Further, the anticancer drug, Adriamycin (Anticancer Res., 20 (2000) 1391), was shown to be effective in such reconstituted systems, the life of which is less than 5 min. In these experiments, the active ingredients of the Ej and LOVO cells were determined on reconstituted sb-BLMs. The key point is that the component part being recognized on the BLM must be kept in its native state.

Keywords: Molecular recognition; Immunoliposomes; sb-BLM; Electrical properties; Anticancer drug; Adriamycin; Planar lipid bilayer; Bilayer lipid membrane

1. Introduction

As a drug carrier and in other applications, liposomes have been studied for many years [1–3]. The main problem of liposomes is that they are mainly taken up by the reticuloendothelial system when administrated in vivo and cannot get to the target sites, which greatly reduces the therapeutic index of medicines, especially for anticancer drugs. One way to overcome this difficulty is by means of immunoliposomes, which are the liposomes linked with the concerned antibody or its fragments. In recent years, several types of immunoliposomes have been developed and have shown promise in this field [4–7]. The difference between conventional

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liposomes and immunoliposomes lies in whether they can be recognized by the receptors of target cells. The molecular recognition will result in a series of physicochemical changes on the lipid membrane, especially changes in electrical properties. In this connection, it has been well established that the bilayer lipid membrane (planar BLM) possesses a similar structure as that of the liposomal membrane, e.g. double phosphatide bilayer [3]. A modified BLM can be a simple model for investigating the molecular recognition occurring on liposomes in vitro. From another point of view, the electrical change of the membrane is sensitive to the specific interactions, such as antigen–antibody, receptor–ligand and enzyme–substrate [3]. This paper presents our attempt at establishing a relationship between the host–guest reaction and its associated electrical change in reconstituted cell membranes for cancer research.

2. Experimental

2.1. Apparatus

The instrument used together with its software data processing was a multifunctional analyzer (Model JI-100, Sino JinKer Electronic Co. Ltd, Tianjin, People's Republic of China), which was connected to a personal computer (SAT, made in People's Republic of China).

2.2. Reagents and materials

The phosphatidylcholine was extracted from eggs and purified according to a well-known procedure [7].The cholesterol was recrystallized (m.p. 147.4–148.1°C) before use. The tumor cells of human bladder, Ej, and rectum, LOVO, were gifts from Prof. Dinner, Alberta University, Canada. The antibody BDI-1 of Ej cells was supplied by Prof. Xie Shusheng, School of Basic Medical Science, Peking University. The other reagents were all of analytical grade and were used without further purification. The materials such as Teflon and plastic tubing and saturated calomel electrodes (SCE) were used as received.

2.3. Procedure

2.3.1. Preparation of agar electrode and salt-bridge-supported BLM (sb-BLM)

The freshly prepared lecithin and cholesterol (4:1, mg/mg lecithin concentration: 20 mg/ml) were dissolved in *n*-decane. The Teflon tube was selected, the diameter of which was 0.5 mm and the length of which was about 2.0–4.0 cm, then sealed at the tip of a thick plastic tube. One end of the tube was about 1 cm in diameter and the other about 1 mm; it was 10 cm long. The Teflon tube was immersed into hot agar solution ($\sim$ 80, 2.5%), and the air in two tubes was excluded through the upper (thick) end, so the surface of hot liquid agar rose slowly. After the thin Teflon tube was filled with agar completely, it was taken out and cooled, then KCl (0.1 M) solution was poured into the plastic tube and one SCE was put in it as an inner reference electrode. According to Ref. [3], the agar-filled tubing was cut at its end with a surgeon's knife and immersed immediately into the BLM-forming solution for 30 s, then taken out to be inserted into 0.1 mol/l KCl, where another SCE was placed as outer reference electrode. The two SCEs were connected to the measuring instrument described above to monitor the change of electrical signals on sb-BLM.

2.3.2. The construction of tumor cell membranes on sb-BLM

The Ej and LOVO cells were each ground, after being cultured to reproduce sufficient amount for experimental need, and mixed with PBS (pH 7.0). The sediment was collected and washed with PBS again after centrifugation at high speed (13 000*g*, for 30 min) and lyophilized ($\sim$ 20, for 48 h). The membrane powder in lipid solution (1 mg/ml) was sonicated briefly and used for reconstituted tumor cell membrane experiments (e.g. Ej–sb-BLM and LOVO–sb-BLM) similar to the conventional sb-BLMs mentioned above.

2.3.3. The antigen–antibody interaction

In the outer KCl solution of the Ej–sb-BLM system, 25, 50 and 100 μl BDI-1 were added, respectively, under the same conditions of addition of 100 μl BDI-1 as control in the LOVO–sb-

BLM system; soon afterwards, the cyclic voltammogram and electrical parameters were recorded.

2.3.4. The interaction of sb-BLM and anticancer drug

To study the influence of the anticancer drug Adriamycin (ADM), 1 mg/ml solution was added dropwise and the change in the BLM was monitored from the screen in the reconstituted LOVO–sb-BLM system. A conventional sb-BLM was used as a control in this experiment.

3. Results and discussion

In the majority of literature available, the preparation of immunoliposomes is based on specific interactions in water solutions (e.g. antigen–antibody, etc.), from which the conclusion is drawn that the same interaction must occur in the lipid surrounding, especially for the aggregation of a quantity of phospholipids (e.g. liposomes). In many cases this is correct, but in many experiments another situation is often seen, in which the recognition between host and guest, which is highly active in water, does not occur or is very weak in liposomes. This may be due to the loss of macromolecular activity during preparation. On the other hand, it is very possible that the reactive ability may change in a different polar medium. In previous experiments, we have only focused attention on the appropriate conditions of preparation; when the immunoliposomes prepared were tested in vivo, the outcome was not ideal. By this time, much manpower and many material resources had been used. How can specific recognition be detected in lipid surrounding before preparing immunoliposomes? The BLM is a very good model, the determination of its electroparameters is direct and straightforward, and the sensitivity of the method is excellent (the concentration of Ag or Ab usually being less than 10^{-9} M). At the same time, the method can be coupled with computer technology, so that online detection may be realized, as will be reported in due course.

3.1. Optimization of sb-BLM

The conventional BLM is formed in a small hole, the boundary of the hole supports the BLM. The c-BLM separates an electrolyte solution into an inner and an outer section and two SCEs inserted in the two sections are linked to the external circuits. The main disadvantage of c-BLMs is their limitation in satisfying the experimental need. First, the forming of a BLM is difficult and time-consuming. Secondly, the BLM is very fragile on account of the disturbance from the bathing solution on both sides of it. Usually the lifetime of a c-BLM is less than 30 min (at best a few hours). The sb-BLM has overcome the instability of the c-BLM, because one side is the interface of aqueous solution/BLM, while the other is that of agar gel/BLM. The sb-BLM can transmit not only electrons but also ions, which are more suitable for biomembrane experiments, because the majority of charged particles conducted are cations and anions, which play an important role in biological processes. The previous method of preparation of sb-BLMs was complex and liable to rupture. In preparing the agar electrode, we have optimized the method reported previously [3]. Compared with the former method, the optimizations are: (a) Ag–AgCl was replaced by SCE because SCE is more commonly used in electrochemistry, since it contains its own salt-bridge; and (b) the inner reference electrode was in solution, not inserted in the agar gel. By reason of these optimizations, the new reference electrode is steadier, and the inner reference electrode need not be glued to the outer layer of the electrode, which is the key point that influences the stability and fidelity in BLM experiments. With these sb-BLMs, typical cyclic voltammograms were obtained, from which the resistance (R_m) and capacitance (C_m) of the membrane were easily calculated. However, unlike the c-BLM, the sb-BLM could not be observed directly through a microscope. The completion of BLM formation was measured using C_m, which reflects the thickness of the membrane, by considering the BLM to be a parallel plate capacitor [8]. The R_m was another principal parameter that depicted the ability of the BLM to conduct electrical charges.

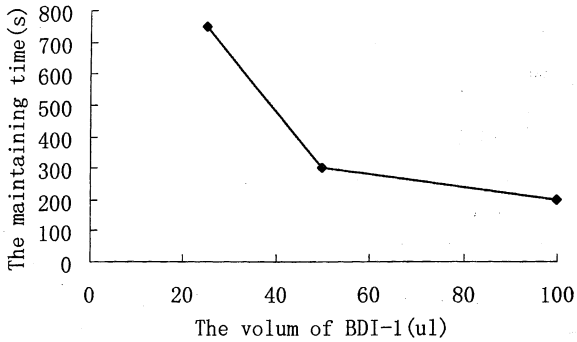

Fig. 1. The lifetime of sb-BLM as a function of added antibody BDI-1. As for LOVO–sb-BLM the BDI-1 added did not affect the membrane.

The change of R_m is related to the interaction led by the ingredient on the BLM and the substance from the bathing solution. When the BLM breaks, R_m reduces exponentially, from which the maintaining time of the BLM can be measured. In our laboratory, the maintaining time of the sb-BLM is often more than 2.5 h, which is much steadier than that of the c-BLM. When the membrane compositions of the Ej and LOVO cells were changed, the lifetime of the reconstituted sb-BLM usually would decrease slightly; however, the value of R_m and C_m did not change significantly.

3.2. Specific interactions

In the Ej–sb-BLM system, the membrane was very fragile and was broken soon after its antibody BDI-1 was added. This may be clearly seen in Fig. 1. As for LOVO–sb-BLM the BDI-1 added did not affect the membrane; the longevity was the same as that of LOVO–sb-BLM without the antibody being added (usually >2500 s). This

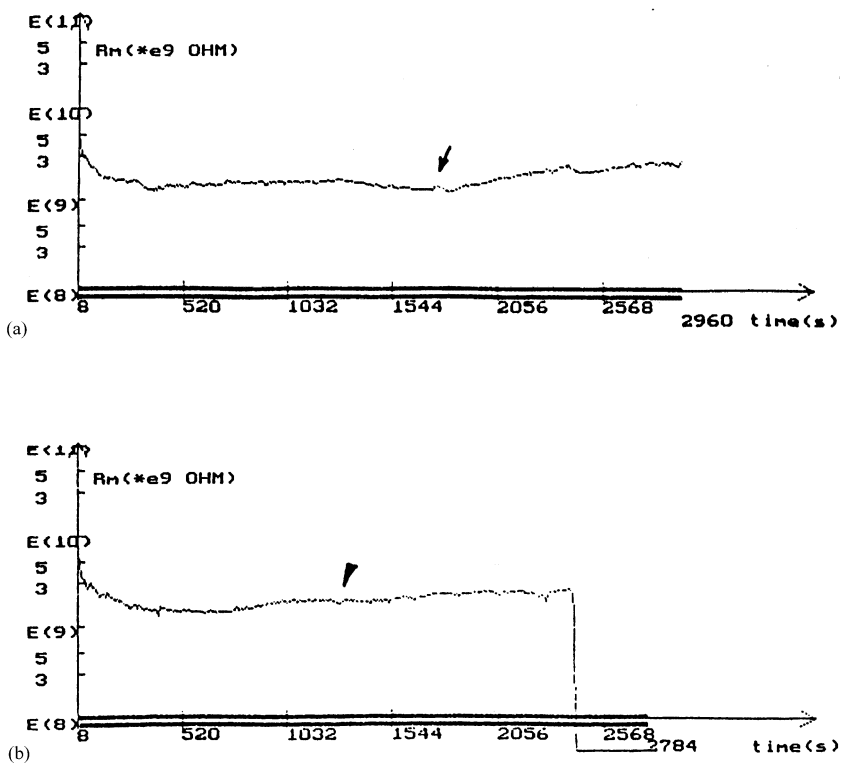

Fig. 2. The longevity of sb-BLM monitored in terms of membrane resistance (R_m). This is illustrated in (a) upper: LOVO-sb-BLM with added antibody (usually >2500s), (b) lower: Ej-sb-BLM with antibody added. The arrows denote the time when the antibody was added.

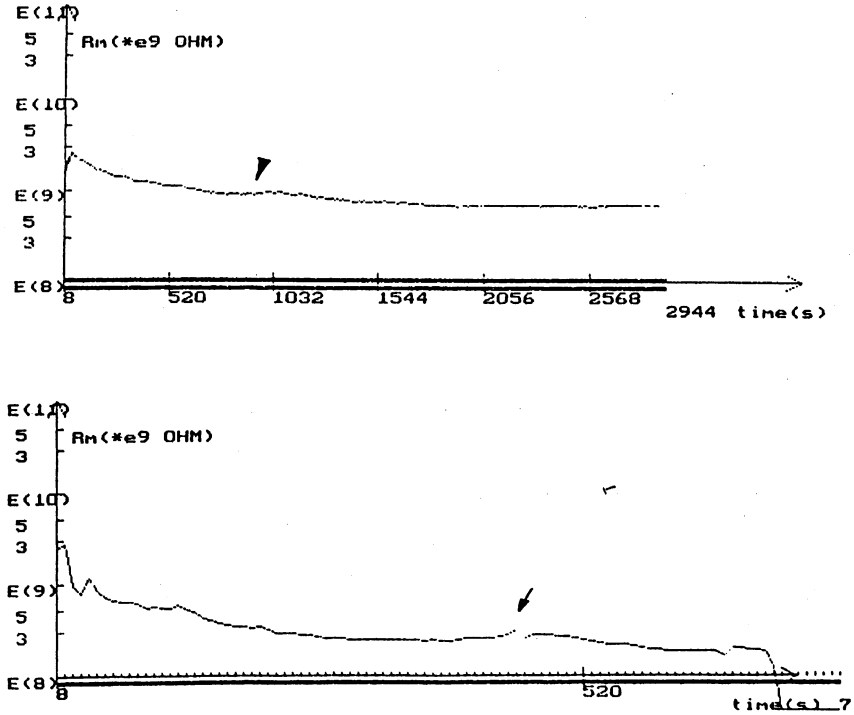

Fig. 3. The longevity of sb-BLM monitored in terms of membrane resistance (R_m) in the presence of ADM to the common sb-BLM: (a) Upper: the membrane did not break until the concentration of ADM reached 0.5%. In contrast, (b) lower: the membrane of LOVO–sb-BLM ruptured when as little as 0.0032% ADM was added, and the maintaining times were all less than 200 s. The arrows denote the time when the antibody or drug was added.

is illustrated in Fig. 2a and b. The arrows denote the time when the antibody or drug was added.

While adding ADM to the common sb-BLM, the membrane did not break until the concentration of ADM reached 0.5%. In contrast, the membrane of LOVO–sb-BLM ruptured when as little as 0.0032% ADM was added, and the maintaining times were all less than 200 s. This is illustrated in Fig. 3a and b. The arrows denote the time when the antibody or drug was added.

These results fully suggested the relationship between the molecular recognition on BLM and the electrical properties (e.g. R_m). For strong interaction of Ej and BDI-1, the life of sb-BLM reduced with the increase in the amount of antibody. In the LOVO–sb-BLM system, there was no such recognition and the membrane could survive for a long time.

The test on ADM and BLM shows that the molecular recognition must have taken place on the reconstituted BLM, but the accurate functional sites are not clear at present. As an effective anticancer drug, ADM generally is deemed to act on the DNA of tumor cells [11]; this experiment revealed another potential mechanism of ADM, namely that the BLM may play a partial role in the antitumor process. In this connection, it has been reported that standard anticancer drugs, ADM (Doxorubicin) and Taxol have been assessed by apoptosis, DNA synthesis, growth rate by MIT assay, uptake of amino acid, and by morphological changes [8–10].

4. Conclusions

This study is aimed at the design of an im-

munoliposome system for molecular recognition using reconstituted, hydrogel-supported bilayer lipid membranes (sb-BLMs). We have shown that, by monitoring the electrical properties, two kinds of recognition are feasible: (i) the human bladder tumor cells, Ej and its antibody BDI-1, the lifetime of the reconstituted membrane is 1000 s; and (ii) the human rectum tumor cells, LOVO, the life of the reconstructed membrane is more than 2500 s, the same as for conventional BLM. Further, the anticancer drug, ADM [11], was shown to be effective in such reconstituted systems, the life of which is less than 240 s.

The specific recognition that occurred on the BLM can be monitored and measured online by its electrical change, which is a possible way of designing and screening effective immunoliposomes in vitro; thus the preparation of immunoliposomes is no longer random. The key point is that the component part being recognized on BLM must be kept in native state. In these experiments, the active ingredients of Ej and LOVO cells were determined on reconstituted sb-BLM in the light of the previous investigation [12]; unlike the phenomena that occurred on c-BLM, the results showed that there was a decrease in R_m and also that the membrane could not be maintained any more; yet, the sb-BLM was more stable than c-BLM, both in theory and practice, which demonstrates the recognition and interaction on BLMs.

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