Characterization of Dyestuff-containing Liposome by Capillary Zone Electrophoresis Using On-line Chemiluminescence Detection

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(Received March 4, 1996)

Eosin Y-containing liposome solution as a sample was migrated and detected by capillary zone electrophoresis for the first time with a chemiluminescence detection using peroxyoxalate system. Two peaks were recorded on the electropherogram; the first one was due to Eosin Y entrapped in liposome and the second one due to free Eosin Y in the bulk solution. The electropherogram comprising the two peaks offered various informations of liposome, such as size distribution, stability, permeability, or surface charge.

Liposome (synthetic lipid bilayer vesicle) has been widely used for model biomembranes, drug carriers, and other purposes. Dyestuff-containing liposome was prepared using calcein, carboxylfluorescein, perylene, etc. and utilized for the characterization (homogeneity, captured volume, stability, permeability, etc.) of liposome, where the dyestuff played an important role as a probe.[1-3] However, the methods for measuring the probe necessitated tedious treatments such as centrifigation, gel filtration, dialysis, etc. and, in general, provided only indirect informations.

On the other hand, the capillary electrophoresis has been recognized as a powerful tool for the separation of various samples. The authors originally developed on-line chemiluminescence (CL) detector for capillary zone electrophoresis (CZE).[4,5] In the CZE-CL method proteins labeled by xanthene dyestuffs were detected through a reaction of bis(2,4,6-trichlorophenyl)oxalate (TCPO)-hydrogen peroxide (H_2O_2) CL system with a high sensitivity.

In this study, the solution of Eosin Y-containing liposome was subjected to the CZE-CL method and two peaks, which were due to Eosin Y entrapped in liposome and free Eosin Y in the bulk solution, were successfully observed on electropherogram. The electropherogram could offer various informations of liposome readily, rapidly, and sensitively. The method is expected to be useful for an investigation of property of liposome.

Multilamellar vesicle (MLV) and small unilamellar vesicle (SUV) were prepared as dyestuff-containing liposome from dipalmitoylphosphatidylcholine (DPPC) and Eosin Y by ordinary methods. EosinY is well known as a common dyestuff to TCPO - H_2O_2 CL system and was used for the CZE-CL method for the determination of protein.[4,5] The apparatus of CZE-CL method developed by us[5] was used for this study. A sample solution of Eosin Y-cotaining lisopome was introduced into a capillary by the siphoning. Analytical conditions are represented in figure captions.

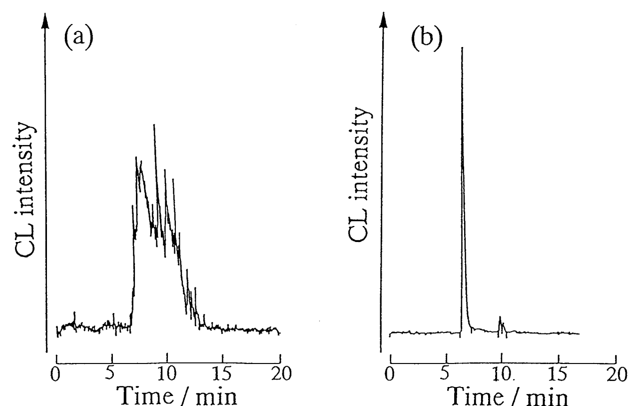

Figure 1. Electropherograms of MLV and SUV as dyestuff-containing liposome.
(a) MLV and (b) SUV.
Conditions: Capillary, 60 cm of 50 μm i. d. fused silica; applied voltage, 20 kV; migration buffer, 10 m mol dm^{-3} carbonate buffer (pH 9.0); CL reagent, a mixture of 1 mmol dm^{-3} TCPO acetonitrile solution of 50 ml and 30 wt% H_2O_2 aqueous solution of 288 μl.

The Eosin Y-containing liposome was migrated to cathode by electroosmotic flow in capillary and was mixed with the CL reagent (TCPO + H_2O_2) at the tip of capillary, where liposome was broken by organic solvent containing the reagent to induce CL signals with a high sensitivity. Under an alkaline condition the free Eosin Y in the bulk solution migrated slower than the liposome due to the negative charge.

Figure 1 shows electropherograms of sample solutions of MLV and SUV. The SUV sample indicated two peaks of Eosin Y entrapped in liposome and free Eosin Y, while the MLV showed notched peaks on the electropherogram. It was reported that size distributions of MLV and SUV were 0.05 - 3 μm[6] and 0.02 - 0.2μm[7], respectively. The difference of size distributions between them must be mainly reflected in these electropherograms.

Sample solutions of SUV were left in 10 mmol dm^{-3} carbonate buffer (pH 9.0) at a room temperature for 1 and 2 day. Their electropherograms were compared together with that obtained immediately after preparation of SUV (Figure 2). The first peak decreased and the second one increased with an increasing of standing time. Furthermore, the influence of standing temperature of SUV on electropherogram was also examined (Figure 3). DPPC has a phase transition point of 41 ℃. At 50 ℃

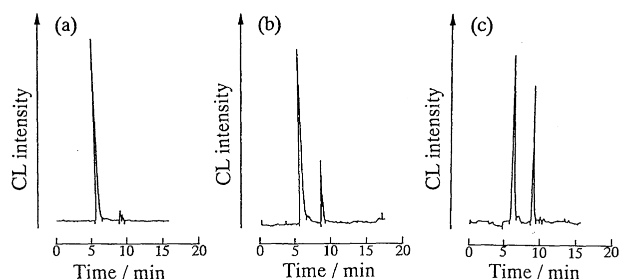

Figure 2. Effect of standing time of SUV as dyestuff-containing liposome on electropherogram.
(a) 0 h, (b) 24 h, and (c) 48 h.
The experiments were carried out under the same conditions as described in Figure 1.

the peak heights changed drastically (the first one decreased and the second increased), while at 25 ℃ the changes were little observed. In the above experiments, changings of the peaks, especially the second peak which was derived from free Eosin Y (what is called a release marker), could easily provide informations of a stability or permeability of liposome.

Another type of Eosin Y-containing liposome was prepared from DPPC and dipalmitoylphosphatidyl-ethanolamine (DPPE) (weight ratio of 10 : 1). When the liposome of DPPC-DPPE was subjected to the present CZE-CL method, liposome or Eosin Y in liposome was recorded at 6.30 min on electropherogram (cf. liposome of DPPC, 5.55 min). DPPC has phosphorus anion and quaternary amine moieties in the molecule, while DPPE has phosphorus anion and primary amine moieties. The liposome of DPPC-DPPE must be modified by more negative charge than that of DPPC under the present conditions. The negative charge would cause the delay of migration time of the liposome.

The measurements of CL signals were performed within 15 min without any pre-treatments of sample solutions, e. g., centrifigation, gel filtration, dialysis. The first and second peak heights of sample solutions left for 1 day were obserbed with coefficients of variations of 4.2 and 2.1% (n=6), respectively. The CL method could detect the second peak about 10 times as sensitive as fluorescent one. Fluorescent method could not detect the first peak, maybe due to a self-quenching of dyestuff. Spectrophotometric detection was, also, not able to be utilized for the detection of the liposome owing to a low sensitivity. The present CL method was only effective to this study.

Recently, drugs[8] or antibodies[9] were labeled by the liposome which entrapped enzymes or dyestuffs for an

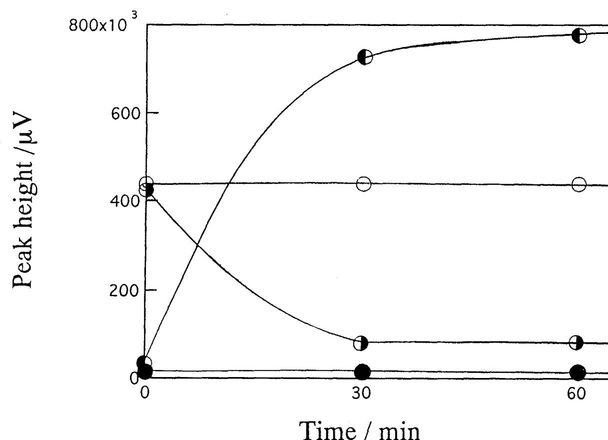

Figure 3. Effect of standing temperature of SUV as dyestuff-containing liposome on peak height.
○ The first peak at 25 ℃, ● the second one at 25 ℃, ◑ the first one at 50 ℃, and ◐ the second one at 50 ℃.
The experiments were carried out under the same conditions as described in Figure 1.

immunoassay. The contents released from the liposome were determined as a release marker by spectrophotometric or fluorescent detection. The results of this report clearly showed that the CZE-CL method is not only effective for the characterization (size distribution, stability, permeability, or surface charge) of liposome but also promising as a means of separation and detection for a new analytical technique such as an immunoassay using liposome.

We thank Mr. A. Arai of Shimazu Corporation for his kind discussion.

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