분류번호	Ⅳ-P-15

Novel Liposome Immunoassay for Detection of Ultratrace Amount of Bioactive Substances : an Assay for Insulin

Soo-Jeong Lim ° and Chong-Kook Kim

Department of Physical Pharmacy, College of Pharmacy, Seoul National University, Seoul, Korea.

The immunoassay method is frequently used for the identification and quantitation of ultratrace amount of bioactive substances. Homogeneous liposome immunoassays, which can avoid the use of radioisotopes and separation steps, have recently been reported in many publications. Cytolysin-mediated liposome immunoassay using melittin ever been studied but showed limited applications. Here, we designed a homogeneous liposome immunoassay using *Clostridium perfringens* phospholipase C (PLC), an enzyme which catalyzes the hydrolysis of phosphatidylcholine in biological membranes, as a cytolysin.

Liposomes incorporated with calcein as a marker were prepared by the reverse-phase evaporation method. The percentage of liposomal lysis by PLC was determined by measuring fluorescence intensity from calcein released out of liposomes. It was affected by the incubation temperature and the composition of liposomes. Based on the optimazation study for measuring lytic activities of PLC, liposomes containing dipalmitoylphosphatodylcholine and cholesterol (9:1) were prepared for performimg a homogeneous liposome immunoassay.

Insulin was selected as a model analyte and conjugated to PLC with heterobifunctional agents. Two types of substrates, liposomal substrate and p-nitrophenylphosphatidylcholine substrate were compared in the immunoassay study. Although conjugate activities against both of them were inhibited by antibody binding on the conjugates, lower concentration of conjugate and short incubation time were required in case of liposomes. In LIA system, the linear range was obtained between 4 and 130 μIU/ml (r = 0.994) and the detection limit was comparable with those of conventional heterogeneous enzyme immunoassays. The LIA method in this study will certainly be useful for increasing the sensitivities of homogeneous immunoassays because liposomes can amplify the signals by entrapping a great number of markers.