

in chloroform:methanol (2:1) was dried down in the bottom of a test tube under a stream of argon. Excess organic solvent was removed by lyophilization overnight. The lipid was dissolved in 600 μ l of 200mM β -D-octylglucoside by vigorous mixing. The lipid-detergent mixture was fractionated through a Sephadex G-25 column (1.5 \times 40 cm) equilibrated in 10mM Tris.HCl, pH 7.6, 60mM NaCl to remove excess detergent and unincorporated 6-carboxyfluorescein. The void volume fractions contained the lipid vesicles with entrapped 6-carboxyfluorescein as identified by monitoring the absorbance at 473 nm. The vesicles were immediately stored at 4° C. under argon.

The release of 6-carboxyfluorescein from the lipid vesicles upon lysis by either detergent or cytolysin (melittin) was monitored by measuring the fluorescence enhancement. Twenty microliters of the vesicle fraction was diluted into 2 ml of 0.15M NaCl, 0.015M sodium phosphate, pH 7.4. The steady state fluorescence was then recorded by exciting at 493 nm and measuring the emission at 519 nm. Addition of excess detergent (0.2% final concentration) or melittin (as little as 20 nanomolar) results in a 3-4 fold increase in fluorescence intensity of the 6-carboxyfluorescein. Vesicle stability was monitored for 28 days with an observed leak rate of 7% 6-carboxyfluorescein released per month.

EXAMPLE 1f

Homogeneous Digoxin Assay

Measurements were performed in a total assay volume of 2 ml of 0.05M Tris HCl, pH 7.8 containing 2 mM p-nitrophenyl phosphate at 37° C. A standard stock solution of digoxin was prepared by dissolving digoxin in dimethylformamide at a concentration of 1 mg/ml. A working dilution of this digoxin stock solution was prepared by dilution to 10 μ g/ml and 1 μ g/ml with 0.05M Tris.HCl, pH 7.8. Final concentrations of 1, 5, and 10 ng/ml digoxin in the assay buffer were achieved by adding 2 μ l, 10 μ l, and 20 μ l of the 1 μ g/ml digoxin to the 2 ml assay solutions, respectively. A final concentration of 100 ng/ml was obtained by adding 20 μ l of the 10 μ g/ml digoxin standard to the 2-ml assay volume and the 1000 ng/ml sample was obtained by adding 2 μ l of the mg/ml digoxin stock solution to 2 ml of assay buffer. After the addition of digoxin, 25 μ l of affinity-purified digoxin antibodies at 6.24×10^{-6} M in phosphate buffered saline were added. Following a one minute preincubation period, 5 μ l of a 7.8×10^{-6} M solution of ouabain-melittin conjugate, as prepared in Example 1d, dissolved in 0.1M sodium acetate, pH 4.5 was added. Following another preincubation period of five minutes, 12 μ l of a 50mM lipid vesicle solution with sequestered alkaline phosphatase in 50mM Tris.HCl, pH 7.8 was added. The absorbancy of this solution was continuously monitored at 410 nm. The kinetic rate of the released enzyme activity ($\Delta A_{410}/\text{min}$) is plotted by a function of final digoxin concentration in the assay mixture as shown in the FIG. 1.

EXAMPLE 2a

Synthesis of Melittin-Biotin Conjugate

Melittin (1.7 mg) was dissolved in 2 ml of 0.15M sodium phosphate, pH 7.8. To this solution was added 5 mg of N-hydroxysuccinimidobiotin, available commercially from a number of suppliers, and the biotinylation reaction was allowed to proceed at 25° C. for 2 hours. During this time, a large precipitate formed. The precipitate was collected by centrifugation and then dis-

solved in 1.5 ml of 0.1M sodium acetate buffer, pH 5.6. This sample was then chromatographed on a Sephadex G-25 column (1.5 \times 40 cm) equilibrated in 0.1M sodium acetate, pH 5.6. The melittin-biotin conjugate was identified in the column eluate by monitoring the absorbance at 280 nm. The peak fractions were pooled and stored at 4° C.

EXAMPLE 2b

Biotin Assay

Vesicles containing entrapped alkaline phosphatase were formed as described in Example 1e. Assays were performed in a total assay volume of 2 mL of 0.05M Tris.HCl, pH 7.8, containing 2mM p-nitrophenylphosphate at 37° C. Free biotin was added to the assay mixtures at concentrations of 0, 0.5, 1, 2, 5, and 10 ng/ml followed by 2 μ l of a 1 mg/ml avidin stock solution (final concentration of avidin was 1.7×10^{-8} M). After 2 minutes at 37° C., 25 μ l of the melittin-biotin conjugate was added (final concentration about 1×10^{-7} M). Five minutes later, 10 μ l of preformed phospholipid vesicles containing entrapped alkaline phosphatase was added and the release of enzyme was followed by monitoring the absorbance change at 410 nm. A plot of enzyme activity versus final biotin concentration in the assay is shown in FIG. 2. Maximum signal was determined by adding 5 ml of a 20% Brij-58 solution to the assay.

EXAMPLE 3a

Synthesis of Melittin-Antibody Conjugate

Monospecific antibodies to the analyte of interest are obtained from high affinity antiserum by affinity chromatography. The procedure for doing this is well known in the art on a specific example is described above in Example 1c. The purified F(ab')₂ fraction is obtained using pepsin digestion and subsequent column chromatography. This methodology is also well known in the art. Briefly, 20 mg of affinity-purified antibodies dissolved in 2 ml of 0.1M sodium acetate buffer, pH 4.5, are digested with 200 μ g of pepsin for 16 hours at 37° C. The sample is clarified by centrifugation and then chromatographed on a 1.5 \times 90 cm column containing Sephadex G-150, a crosslinked beaded dextran for gel permeation chromatography, equilibrated in 0.015M sodium phosphate, pH 7.4, 0.15M NaCl. The column fractions containing the F(ab')₂ fragments, identified by gel electrophoresis, are pooled and reconcentrated to 2 ml by pressure filtration. The F(ab')₂ fragments are then converted to their corresponding monovalent Fab' fragments by reductive cleavage with dithiothreitol. To do this, the solution of F(ab')₂-fragments from above are made 20mM in dithiothreitol and allowed to incubate at 25° C. for 1½ hours under an atmosphere of argon. Excess dithiothreitol is removed by dialysis against 0.1M sodium phosphate, pH 5.6 at 4° C.

The Fab'-fragments are coupled to melittin using the heterobifunctional crosslinking agent m-maleimidobenzoyl N-hydroxysuccinimide ester (MBS, purchased from Pierce Chemicals). In this way the amino residues of melittin are acylated by the active N-hydroxysuccinimidyl ester of MBS to form a covalent adduct, and then the free sulfhydryl groups on the Fab'-fragments form a covalent adduct with the maleimidyl moiety of MBS.

For this, 2 mg of melittin (6.7×10^{-7} mols) dissolved in 1 ml of phosphate buffered saline, pH 7.0, is mixed