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Protein G-liposomal nanovesicles as universal reagents for immunoassays

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Abstract

To improve the antigen-binding activity of liposome-coupled antibodies and to develop universal liposomal nanovesicles for immunoassays, protein G was conjugated to dye-loaded liposomal nanovesicles for the preparation of immunoliposomes. Sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), a heterobifunctional cross-linker, was used to modify protein G for conjugation to the liposomal nanovesicles. Liposome immunosorbent assays were used to evaluate the binding ability of protein G after sulfo-SMCC modification, to optimize the protein G density on the liposome surface and to determine the amount of IgG binding to the protein G-liposomal nanovesicles. Test strips coated with a narrow zone of antibodies were used to show the successful conjugation. Immunomagnetic beads were used to demonstrate the feasibility of protein G-tagged universal liposomal nanovesicles for immunoassays. Results indicate that the Fc-binding capacity of protein G decreased by only 5.3% after sulfo-SMCC modification. Antibodies were easily conjugated to universal protein G-liposomal nanovesicles in 30 min. The conjugates (protein G-immunoliposomes) were successfully used in immunomagnetic bead assays for the detection of *Escherichia coli* O157:H7 with a detection limit of approximately 100 CFU/ml. This work demonstrated that protein G-liposomal nanovesicles are a successful universal reagent for easily coupling antibodies in an active orientation on the liposome surface for use in immunoassays.

Keywords: Protein G; Liposome; Nanovesicle; Universal reagent; Immunoassay

1. Introduction

Liposomal nanovesicles, i.e., liposomes, are spherical vesicles consisting of phospholipid bilayers surrounding an aqueous cavity. Because each liposomal nanovesicle can contain up to several million fluorescent dye molecules, thereby providing greatly enhanced signals, antibody-tagged liposomal nanovesicles (immunoliposomes) have been successfully used as reporter particles in immunoassays [1–4]. The strategies for conjugating antibodies to liposomal nanovesicles involve mostly covalent binding using cross-linking molecules, such as *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), *N*-succinimidyl-S-acetylthioacetate (SATA), succinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) and succinimidyl-4-(*p*-maleimidophenyl)butyrate (SMPB) [5–8]. The noncovalent biotin–(strept)avidin coupling method also involves the biotinylation of antibodies [9]. These methods generally require the use of the amino groups on the antibody. This approach, however, is limited because most antibodies contain randomly distributed amino groups, leading to multiple attachment sites. The random nature of this attachment can cause some of the conjugated antibodies to lose antigen-binding activity due to direct chemical modification or steric hindrance of the antigen-binding site [10,11].

To improve the antigen-binding activity of liposome-tagging antibodies, IgG Fc-binding proteins, like protein A or protein G, can be used to couple antibodies to liposomal

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nanovesicles in an oriented manner. Protein A is a bacterial cell wall protein isolated from *Staphylococcus aureus*. Protein G is also a cell wall protein obtained from group G *Streptococci*. While both of these proteins can be used for binding antibodies, compared with protein A, protein G represents a more general and versatile IgG binding reagent [12]. It binds a wider range of IgG subclasses and a greater variety of mammalian species with higher affinity than protein A. In addition, protein G is not as pH dependent as protein A when binding to immunoglobulins [12–14].

One of our objectives was to improve the antigen-binding activity of conjugated antibodies by first tagging the liposomal nanovesicle with protein G to specifically orient the antigen-binding site of the antibodies away from the liposome surface. Our other objective was to show that protein G-liposomal nanovesicles can be successfully used as universal reagents for immunoassays. While it is very complicated and time-consuming to covalently conjugate antibodies to the liposome surface, protein G-liposomal nanovesicles should couple antibodies quickly and easily.

In this study, sulfo-SMCC, a heterobifunctional crosslinker, was used to modify protein G for conjugation to the sulfhydryl group-containing surface of dye-loaded liposomal nanovesicles. Also, liposome immunosorbent assays were used to evaluate the binding ability of the modified protein G, to optimize the protein G density on the liposome surface and to determine the amount of IgG binding to protein G-liposomal nanovesicles. After covalently conjugating protein G to the dye-loaded liposomal nanovesicles, lateral flow test strips with antibodies bound in a narrow zone were used to show the successful conjugation. We also demonstrated the feasibility of using universal protein G-liposomal nanovesicles by detecting *Escherichia coli* O157:H7 in immunomagnetic bead assays.

2. Experimental

2.1. Reagents

Common laboratory reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). Dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG) and polycarbonate syringe filters of 0.4 and 0.2 μm pore sizes were purchased from Avanti Polar Lipids (Alabaster, AL). Dipalmitoylphosphatidylethanolamine (DPPE) was purchased from Molecular Probes (Eugene, OR). *N*-Succinimidyl-*S*-acetylthioacetate, protein G (recombinant, *Streptococcus*), Blocker Casein in TBS and sulfo-SMCC were purchased from Pierce (Rockford, IL). Immunomagnetic beads against *E. coli* O157:H7 were purchased from Neogen (Lansing, MI). Antibodies against *E. coli* O157:H7 were purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD). Antibodies (AffiniPure Goat Anti-Rabbit IgG) coated on test strips and ChromPure® Goat IgG, whole molecule (from normal sera) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). *E. coli* O157:H7 strain 43895 was kindly provided by Randy Worobo and John Churey, Cornell University, Geneva, NY. Predator® membrane (Pall Corporation, Port Washington, NY) test strips coated with a narrow zone of antibodies were kindly provided by Thomas DeCory in our research group.

2.2. Preparation of acetylthioacetate (ATA)-tagged liposomes containing sulforhodamine B (SRB)

The liposome encapsulant, a 150 mM SRB solution, was prepared in 0.02 M HEPES buffer (pH 7.5, 496 mosmol/kg). A solution was prepared containing 7.2 μmol of DPPE and a volume fraction of 0.7% triethylamine in chloroform. This solution was reacted with 14.3 μmol of SATA to form DPPE-acetylthioacetate (DPPE-ATA). Then 40.3 μmol of DPPC, 4.2 μmol of DPPG and 40.9 μmol of cholesterol were dissolved in a solvent mixture consisting of 3 ml of chloroform and 1 ml of methanol. To this lipid solution, 3.6 μmol of DPPE-ATA was added and mixed thoroughly. Using vacuum rotary evaporation, the organic solvent was removed yielding a thin dry lipid film. Three milliliters of the SRB encapsulant was then added to hydrate the lipid mixture. The hydrated lipid suspension was subjected to five freeze/thaw cycles by alternately placing the sample vial in a dry ice/acetone bath and 50 °C water bath. Once the lipid mixture was fully hydrated, it was allowed to stand for 4 h at 50 °C. Finally, the liposomes were extruded through polycarbonate syringe filters with 0.4 and 0.2 μm pore size in series. To remove unencapsulated dye, the liposomes were gel filtered on a Sephadex G-50-150 column. The liposomes were stored at 4 °C in Tris-buffered saline (0.02 M Tris, 0.2 M NaCl, 0.01% sodium azide, pH 7.5, 550 mosmol/kg).

2.3. Conjugation of protein G to ATA-tagged liposomes

Protein G (dissolved in 0.05 M phosphate buffer, pH 7.8, containing 1 mM EDTA) was modified with a maleimide group by incubation with 15 times the molar quantity of sulfo-SMCC dissolved in dimethyl sulfoxide (DMSO). The reagents were allowed to react for 2 h at room temperature. Hydroxylamine hydrochloride was used to deacetylate the ATA groups on the liposome to yield the reactive sulfhydryl groups. For this reaction, 0.5 M hydroxylamine hydrochloride solution with 25 mM EDTA in 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid) buffer (pH 7.5) was prepared and 0.1 ml of this solution was added per 1 ml of the liposome solution. The reaction was allowed to proceed at room temperature in the dark for 2 h. For conjugation, the thiol groups on the liposome surface were reacted with the maleimide group-modified protein G at pH 7 for 3.5 h at room temperature and then overnight at 4 °C. All unconjugated thiol groups were quenched with ethylmaleimide solution, which is isotonic to the encapsulant. The protein G-liposomal nanovesicles were then purified by size-exclusion

chromatography using Sepharose CL-4B equilibrated with Tris-buffered saline (0.02 M Tris, 0.2 M NaCl, 0.01% sodium azide, pH 7.5, 550 mosmol/kg).

2.4. Conjugation of IgG to ATA-tagged liposomes

For evaluating protein G binding ability by using a liposome immunosorbent assay, we conjugated IgG to liposomes as conventional immunoliposomes. The protocol was the same as that described above for conjugating protein G to ATA-tagged liposomes.

2.5. Evaluation of protein G binding ability after sulfo-SMCC modification

We used competitive liposome immunosorbent assays to evaluate the binding ability of protein G after sulfo-SMCC modification. Microtiter plates (PolySorp, Nunc, Rochester, NY) were coated overnight at 4 °C with 200 μl of 20 μg/ml protein G dissolved in 0.05 M phosphate buffer, pH 7.8, containing 1 mM EDTA. Here, the protein G served as a surface-bound capture agent for conventional nonoriented immunoliposomes in competition with dissolved modified or unmodified protein G. After removing excess protein G, plates were blocked at room temperature for 30 min with Blocker Casein in TBS (25 mM Tris, 150 mM NaCl, pH 7.4). Plates were then washed three times with Blocker Casein in TBS with 0.05% Tween-20 (BCT). Nonoriented immunoliposomes made by the method described above were mixed with 2.5 μg/ml of modified or unmodified protein G. The mixtures were added to test and control wells and incubated for 1 h, gently shaken at room temperature. Unbound immunoliposomes were removed and wells were then washed three times with BCT. To lyse the bound immunoliposomes, 200 μl of 30 mM *n*-octyl-β-D-glucopyranoside (*n*-OG) was added to each well and shaken at room temperature for 5 min. The fluorescence signal was read in a fluorescence plate reader (Cytofluor, PerSeptive Biosystems) using an excitation filter of 530 nm and emission filter of 590 nm.

2.6. Lateral-flow test strip assays

In a 10 mm × 75 mm glass test tube, 4 μl of liposome solution was mixed with 3 μl of Blocker Casein in TBS and then the Predator® membrane test strip coated with a narrow zone of antibodies (AffiniPure Goat Anti-Rabbit IgG) was inserted. After the mixture was absorbed into the test strip, 40 μl of Blocker Casein in TBS was added into the test tube. The strip was left in the tube until all of the solution was drawn from the bottom of the test tube.

2.7. Optimization of the protein G density on the liposome surface

Liposome immunosorbent assays were used to evaluate the IgG-binding ability of the protein G-liposomal nanovesicles with different protein G surface densities. PolySorp microtiter plates were coated overnight at 4 °C with 200 μl of 20 μg/ml ChromPure® Goat IgG dissolved in 0.05 M phosphate buffer, pH 7.8, containing 1 mM EDTA. Here, the IgG served as a surface-bound capture agent for protein G-liposomal nanovesicles. After removing excess IgG, plates were blocked at room temperature for 30 min with Blocker Casein in TBS (25 mM Tris, 150 mM NaCl, pH 7.4). Plates were then washed three times with BCT. Protein G-liposomal nanovesicles with 0.07, 0.15, 0.3, 0.4, 0.6, 0.8 mole% tag were added to the test and control wells and incubated for 1 h, gently shaking at room temperature. Unbound protein G-liposomal nanovesicles were removed and the wells were then washed three times with BCT. To lyse the bound protein G-liposomal nanovesicles, 200 μl of 30 mM *n*-OG was added to each well and shaken at room temperature for 5 min. The fluorescence signal was read in a Cytofluor fluorescence plate reader as before.

2.8. Optimization of the amount of IgG binding to protein G-liposomal nanovesicles

Liposome immunosorbent assays were also used to optimize the amount of IgG binding to protein G-liposomal nanovesicles. Microtiter plates (PolySorp) were coated overnight at 4 °C with 200 μl of 20 μg/ml ChromPure® Goat IgG dissolved in 0.05 M phosphate buffer, pH 7.8, containing 1 mM EDTA. Here, the IgG served as a surface-bound capture agent for protein G-liposomal nanovesicles. After removing excess IgG, plates were blocked at room temperature for 30 min with Blocker Casein in TBS. Plates were then washed three times with BCT. Protein G-liposomal nanovesicles were mixed with different amounts of IgG against *E. coli* O157:H7 for 30 min. The mixtures were then added to the test and control wells and incubated for 1 h, gently shaking at room temperature. Unbound protein G-immunoliposomes were removed and the wells were then washed three times with BCT. The bound protein G-immunoliposomes were lysed with 200 μl of 30 mM *n*-OG and the fluorescence signal was read in a Cytofluor fluorescence plate reader as before.

2.9. Coupling antibodies to protein G-liposomal nanovesicles

Antibodies (dissolved in 0.05 M phosphate buffer, pH 7.8, containing 1 mM EDTA) were added to the protein G-tagged liposome solution and incubated for 30 min at room temperature. Unbound antibodies were removed by gel filtration on Sepharose CL-4B.

2.10. Bacterial inoculum preparation

After *E. coli* O157:H7 was inoculated into tryptic soy broth and incubated for 18 h at 37 °C, it was serially diluted with TBS buffer. The population of bacteria in the dilution

tubes was determined by spread plate counts in triplicate on tryptic soy agar.

2.11. Immunomagnetic bead assays

Two microliters of immunomagnetic bead stock solution was added to 3 ml of serially diluted culture. After 1 h incubation at room temperature on a Labquake rotator (Barnstead/Thermolyne, Dubuque, IA), samples were placed in a magnetic particle separator for 3 min and the supernatant was discarded. The beads were washed twice in BCT. Ten microliters of protein G-immunoliposome solution and 190 μl of Blocker Casein in TBS were added and incubated for 30 min at room temperature. The magnetic beads were separated and washed three times in BCT. They were re-suspended in 200 μl of 30 mM *n*-OG and vigorously vortexed. After the beads were magnetically separated, the fluorescence signal of the supernatant was read in an AquaFluor Handheld Fluorometer (Turner Designs, Sunnyvale, CA).

3. Results and discussion

3.1. Protein G modification for conjugation with liposomal nanovesicles

The procedure for preparing protein G-liposomal nanovesicles involved the activation of the liposome surface with sulfhydryl groups and allowing them to react with maleimide groups derivatized on protein G. This was achieved by reacting DPPE with SATA, forming DPPE-ATA, which was incorporated into the liposomes. Protein G was modified with maleimide groups by sulfo-SMCC, whereby the sulfosuccinimidyl groups react with primary amines on the protein G. Since the IgG Fc-binding domain of protein G has primary amines, sulfo-SMCC may cause direct modification on the Fc-binding domain and lead to the loss of protein G Fc-binding activity. Therefore, we developed competitive liposome immunosorbent assays to evaluate the IgG Fc-binding ability of protein G after sulfo-SMCC modification.

Protein G coated in the well of a microtiter plate by noncovalent adsorption served as a surface-bound capture agent for conventional, nonoriented immunoliposomes in competition with tested modified or unmodified protein G. Due to the exposure of the IgG Fc fragment on the nonoriented immunoliposomes, they have the ability to bind protein G. The data given in Table 1 demonstrate that unmodified protein G inhibits 89.9% of immunoliposomes binding to protein G in the well and modified protein G of the same concentration inhibits 84.6%. Thus, the IgG Fc-binding capacity of protein G decreased only slightly after sulfo-SMCC modification.

One of the possible explanations for only a slight decrease is that protein G has two Fc-binding domains. It has been proposed that in the eight residues most involved in Fc-binding, only two of them are lysine residues which provide primary amines at the surface of protein G. Furthermore, the two lysine residues are located in the same domain [15]. Therefore, even if the two lysines were both reacted with sulfo-SMCC, the other Fc-binding domain of protein G is still available for binding IgG.

Table 1
Competitive inhibition of immunoliposomes binding to protein G in the microtiter well by protein G or modified protein G (protein G′)

	Fluorescence (au)	Inhibition (%)
Protein G	64	89.9
Protein G′	98	84.6
Positive control	636	0
Negative control	11	100

Positive control, no protein G or protein G′ was mixed with immunoliposomes. Negative control, no protein G was coated in the wells. Inhibition values were corrected for the negative control background fluorescence.

3.2. Lateral-flow test strip assays

Test strip assays were used to show the successful conjugation of protein G to liposomes and IgG to protein G-liposomal nanovesicles. This test strip assay format is based on protein G-directed binding of liposomes to IgGs, capillary migration on the test strip and detection in the antibody zone. If binding occurs between protein G-liposomal nanovesicles and the immobilized antibodies in the narrow antibody zone, a colored band appears in the zone. Sample components that do not bind to the immobilized antibodies in this zone migrate to the end of the test strip and leave no colored band in the zone.

Fig. 1 shows that protein G was successfully coupled to the liposomes because only sample B formed a visible band in the narrow zone by the binding between IgG on the strip and protein G on the liposomes. These four test strips were identical and coated with IgG in the narrow zones. The lipo-

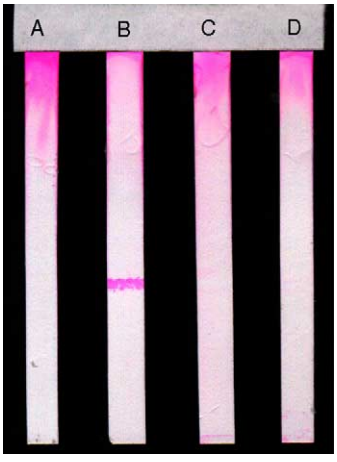

Fig. 1. Scanned image of test strips showing the protein G-liposomal nanovesicles binding to immobilized IgGs. Sample A contains ATA-tagged liposomes; sample B, protein G-liposomal nanovesicles; sample C, IgG-tagged liposomes; sample D, mixture of IgGs and protein G-liposomal nanovesicles, pre-incubated for 30 min.

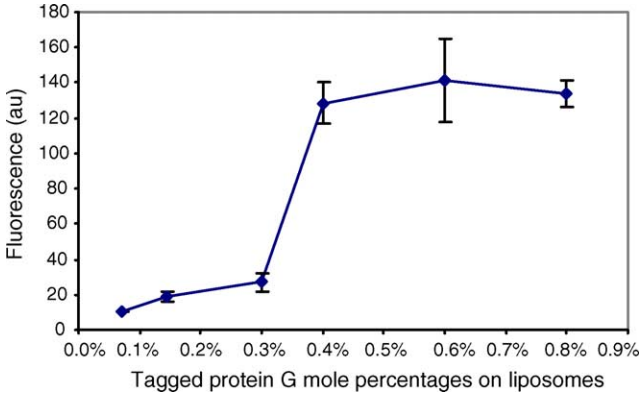

Fig. 2. IgG-binding ability of different amounts of protein G for coupling to liposomes.

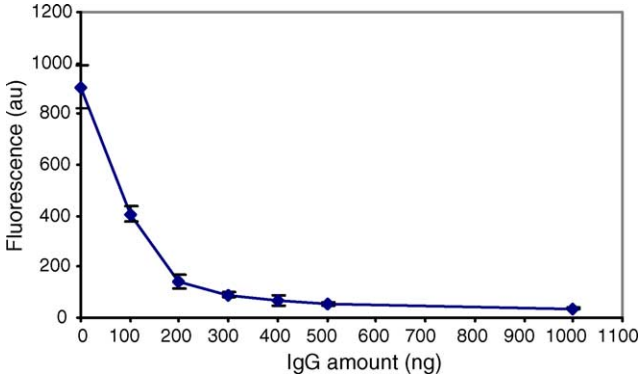

Fig. 3. Competitive inhibition of protein G-liposomal nanovesicles binding to IgGs in the microtiter plate well by IgGs against *Escherichia coli* O157:H7.

somes of samples A and B were both made from ATA-tagged liposomes. Sample B liposomes were subsequently conjugated to protein G. Neither the ATA-tagged liposomes nor the IgG-tagged liposomes (sample C) formed a band. Also, after a sufficient amount of IgG was mixed with protein G-liposomal nanovesicles for 30 min, the mixture (sample D) could not form a band on the test strip. It shows that IgG was successfully conjugated to protein G-liposomal nanovesicles in 30 min, thereby preventing protein G from binding to the immobilized antibodies in the test strip. Considering the complex and time-consuming (at least 2 days) process required to prepare conventional immunoliposomes, protein G-tagged universal liposomes provided a much simpler and faster way to couple antibodies.

3.3. Optimization of protein G-liposomal nanovesicles

The protein G density on the liposome surface was optimized to achieve maximum sensitivity. Initially, different added amounts of protein G for conjugation to liposomes were tested with 0.07, 0.15, 0.3, 0.4, 0.6, 0.8 mole% tags. We used liposome immunosorbent assays to investigate the IgG-binding ability of those liposomes with different protein G densities. IgG coated on a microtiter plate served as a surface-bound capture agent for those protein G-liposomal nanovesicles. The higher the fluorescent signal from the assay, the stronger the IgG-binding ability. The results show that the fluorescent signal increased with increasing density of protein G on the liposome surface from 0.07% to 0.4% and remained approximately the same above 0.4% (Fig. 2). Therefore, the 0.4% tag was selected for making the protein G-liposomal nanovesicles.

3.4. Optimization of the amount of IgG binding to protein G-liposomal nanovesicles

Since too few IgGs binding to protein G-liposomal nanovesicles will cause a high background signal in sandwich assays and also to conserve the supply of IgGs, optimization of the IgG amount was necessary. Liposome immunosorbent assays were used to evaluate the IgG-binding ability of protein G-immunoliposomes after mixing protein G-liposomal nanovesicles and IgG against *E. coli* O157:H7 for 30 min. Based on the IgG-binding ability of protein G-immunoliposomes, the optimum amount of IgG amount for protein G-immunoliposomes was determined. Fig. 3 shows that, as expected, the greater the amount of IgGs added to protein G-liposomal nanovesicles, the lower is the IgG-binding ability of the protein G-immunoliposomes. Theoretically, we should make protein G-immunoliposomes having no residual IgG-binding ability to avoid the background signal in sandwich assays. However, as seen in Fig. 3, that will require very large amounts of IgG. We found that 200 ng of IgG inhibited 85% of the IgG-binding ability of 1 μl protein G-liposomal nanovesicles, 300 ng inhibited 90% and 500 ng inhibited almost 95%. In other words, 500 ng of IgG saturated 95% of the protein G binding sites on the liposomes. Since increasing amounts did not significantly improve saturation, it was decided to use 500 ng of IgG per microliter of protein G-liposomal nanovesicles.

3.5. Immunomagnetic bead assays for E. coli O157:H7

For demonstrating the analytical feasibility of the protein G-liposomal nanovesicles, immunomagnetic bead assays were used to detect *E. coli* O157:H7. The multivalent bacterium binds in sandwich fashion between the immunomagnetic beads and protein G-immunoliposomes. The sandwich structure complexes are magnetically separated from the sample matrix and unbound liposomes and then the bound liposomes are lysed by a detergent solution. Finally, the level of bacteria in the sample is quantified by measuring the fluorescence intensity of the fluorescent dye molecules released by the lysis.

The analytical sensitivity and detection limit were determined from the dose–response curve (Fig. 4a and b). The limit of detection (LOD) is defined as the lowest concentration of analyte producing a fluorescence intensity that is 3 S.D. higher than the mean intensity at zero concentration (negative control). According to this definition, the LOD of this assay

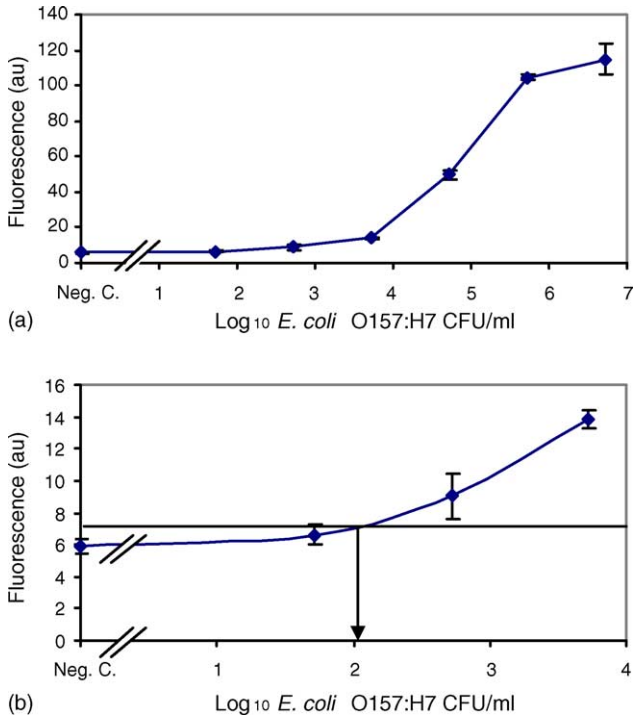

Fig. 4. (a) Dose–response curve of *E. coli* O157:H7 in an immunomagnetic bead assay using protein G-immunoliposomes. Error bars represent the standard deviations of triplicate measurements. (b) Expanded dose–response curve of (a) showing location of the LOD. The straight horizontal line is 3 S.D. higher than the mean intensity at zero concentration (negative control) and the intersection of this line with the dose–response curve is the LOD as indicated by the vertical arrow.

for *E. coli* O157:H7 was determined to be approximately 100 CFU/ml (Fig. 4b). As seen in the full dose–response curve (Fig. 4a), the fluorescent signal increases with increasing concentration of *E. coli* O157:H7, showing a broad sigmoidal dynamic range over four orders of magnitude from ca. 10^2 to 10^6 CFU/ml and greatly increasing in sensitivity above about 10^4 CFU/ml. Also, only 1/10 of the manufacturer's suggested amount of immunomagnetic beads (20 μl) were required for the assay and the entire assay could be completed in about 2 h without the need for enrichment or membrane filtration.

These results suggest that this assay is comparable to, or better than, other currently available detection assays for *E. coli* O157:H7. The detection limit of an enzyme-linked immunosorbent assay (ELISA) was reported to be approximately 10^5 CFU/ml and the dynamic range was from 10^5 to 10^7 CFU/ml [16]. A fluorescent bacteriophage assay was capable of detecting 10^4 CFU/ml [17]. Several research groups also developed assays using immunomagnetic beads for *E. coli* O157:H7. For example, Seo et al. described a method combining immunomagnetic separation and flow cytometry. Their detection limit was 10^3 cells/ml [18]. Perez et al. developed a 2-h assay incorporating immunomagnetic beads in an amperometric flow injection system with the detection limit of 10^5 CFU/ml [19]. Yu et al. used a system based on immunomagnetic separation and time-resolved fluorometry and was able to detect 10^3 *E. coli* O157:H7 per milliliter [20]. Yu and Bruno reported a detection limit of 10^2 bacteria per milliliter in the immunomagnetic–electrochemiluminescent detection of *E. coli* O157:H7 [21]. The high sensitivity in our study is in part attributable to the advantages offered by dye-encapsulating liposomes. Because each liposome contains a large numbers of fluorescent dye molecules, the signals generated from the binding events are greatly enhanced, thereby resulting in higher sensitivity. This study demonstrates that protein G-liposomal nanovesicles are successful reagents for immunoassays.

3.6. Comparison between conventional immunoliposomes and protein G-immunoliposomes

By using the same liposome concentration for both types of immunoliposomes in immunomagnetic bead assays, no significant difference in the fluorescent intensity was observed for the same concentrations of *E. coli* O157:H7 (data not shown). However, for preparing the conventional immunoliposomes, 0.6 μg of IgG per nanomole of total lipid was used in comparison to 0.28 μg of IgG per nanomole of total lipid for making protein G-immunoliposomes. This lower surface density of the IgG demonstrated that protein G makes immunoliposomes more efficient by coupling the antibodies in an active orientation on the liposome surface.

4. Conclusion

The antibody coupling to protein G-liposomal nanovesicles was completed in only 30 min, which is much faster than the covalent method for the preparation of conventional immunoliposomes. The immunomagnetic bead-immunoliposome assays performed by protein G-liposomal nanovesicles showed sensitivities comparable to other detection assays. These results demonstrated that protein G-liposomal nanovesicles are successful universal reagents for easily coupling antibodies in an active orientation on the liposome surface for use in immunoassays. In this study, immunomagnetic bead assays for *E. coli* O157:H7 demonstrated the feasibility of using protein G-liposomal nanovesicles. In the future, assays for other pathogens and the feasibility of using protein G-liposomal nanovesicles in other assay formats will be studied.

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