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Use of Antibody Displayed Phage for the Detection of Dextran Using a Dipstick Assay and Transmission Electron Micrograph

KIM, DUWOON* AND DONAL F. DAY[1]

Department of Food and Nutrition, Chonnam National University, Chonnam 550-749, Korea
[1]The Audubon Sugar Institute, LSU Agricultural Experiment Station, 3845 HWY 75, St. Gabriel, LA 70776, U.S.A.

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Abstract An antibody displayed phage collection (SBAE-2R), screened from a human synthetic phage antibody library (Fab 2lox), was used for the determination of dextran. The dextran-binding affinity was determined by serologically specific transmission electron microscopy (TEM) and a paper dipstick assay. The phage collection was distributed over the dextran-coated grids with 39±25 phages/μm^2 on the grids. Phages were not seen on dextran-coated grids exposed to the Fab 2lox phage library. The phage collection (SBAE-2R) produced 54±3 color normalized intensity (N.I.) from 125 ppm to 1,000 ppm of dextran and 5±1 (N.I.) for 63 ppm of dextran in a paper dipstick assay. This research extends the analytical options for dextran analysis by antibody displayed phage with a minimum of equipment usage.

Key words: Dextran, electron microscopy, phage display

A phage display antibody library contains all possible antibody fragments as inserts in a viral coat protein. Use of a library simplifies the process for production of antigen-binding proteins [5, 10]. Use of these libraries allows selection of antigen-binding proteins without animal immunization or hybridoma production [3, 6]. A filamentous phage (Fd) displays Fab (antigen binding fragments) as fusion proteins with a minor coat protein (pIII). Antibody fragments (Fab) expressed on the surface of this filamentous bacteriophage can be used as reagents for detecting antigens, tumor cells, viruses, and toxins. Numerous applications have been reported on the use of Fab fragments [7], including immunotherapy, delivery of molecules to kill specific cells, neutralization of HIV-2, vaccine development, and the activation of T cells around tumor cells [1, 2, 10]. Quantitative assay using serologically specific electron microscopy (SSEM) was used as a diagnostic method to detect virus in the crude extract, where a specific virus was attached to electron microscope grids coated with an adsorbed film of antivirus antibody [8]. Dipstick assays have the advantages of simplicity, rapidity, and low cost. Clinical diagnostic applications of dipstick tests include detection of adenovirus, Chlamydia, influenza A and B, Mycoplasma, and *Bordetella pertussis* [15, 16]. In this study, our approach on the use of transmission electron microscopy and a dipstick as a diagnostic assay are given to determine the dextran-binding affinity of phage collection (SBAE-2R) enriched from Sephadex bead-agarose electrophoresis (SBAE) [12].

A human synthetic phage-antibody library (Human Synthetic Fab 2lox Library) was acquired from the Cambridge Centre for Protein Engineering, England. This library contains a large (1.2×10^{12}) synthetic repertoire of Fab fragments [13]. A dipstick assay was used to show the dextran-binding affinity, as described by Kim and Day [12]. Fab DNA amplification was conducted at 94°C, with 5 min for presoaking, followed by 30 cycles of 94°C, 1 min for denaturation at 60°C, 1 min for annealing at 72°C, 1.5 min for extension, and finished by incubation at 72°C for 5 min in 50 µl of PCR reaction mixture with two primers, 10 pmol/µl of Fdpcrback-1 (GCGATGGTTGTTGTCATT) and ten pmol/ml of Fdseq1Forward (CCTCATACAGAAAATTC). The PCR products were separated by electrophoresis at 70 volts for 60 min on 0.8 % agarose gels using TAE buffer [4, 10, 13, 16]. Phage collection (SBAE-2R) enriched by Kim and Day [2] was used to visualize the binding of phage to dextran by transmission electron microscopy (TEM), as described by modification of the method of Derrick and Brlansky [8]. Copper TEM specimen grids coated with collodion and carbon were placed for 5 min on drops (10 µl) of mouse monoclonal anti-dextran antibody, diluted 1:500 with sterile distilled water. Excess anti-dextran

*Corresponding author
Phone: 82-61-659-2953; Fax: 82-61-655-0490;
E-mail: dwkim@chonnam.ac.kr

antibody was washed off the grids by touching each to 10-μl drops of distilled water, twice. The copper TEM specimen grids were then placed for 1 min on drops (10 μl) of dextran (T10, 500 ppm) dissolved in filtered water, air dried for 5 min, blotted with filter paper to drain excess solution, and then touched to a 20-μl drop of test (SBAE-2R) or control phage (Fab 2lox library) solution (10^5 transducing unit, t.u./ml) and rinsed once with filtered water. The grids were exposed to 50 μl of 2% uranyl acetate negative stain, drained, and then imaged with a JEOL 100CX TEM operating at 80 kV (The Socolofsky Microscopy Center, Louisiana State University at Baton Rouge, LA, U.S.A.). Test (SBAE-2R) or control (Fab 2lox library) phages were quantitated by counting phages per μm^2 TEM images obtained from forty-eight fields on 8 grids. Control phage (Fab 2lox library) was used to calculate the degree of the nonspecific binding on the copper TEM grids coated with dextran.

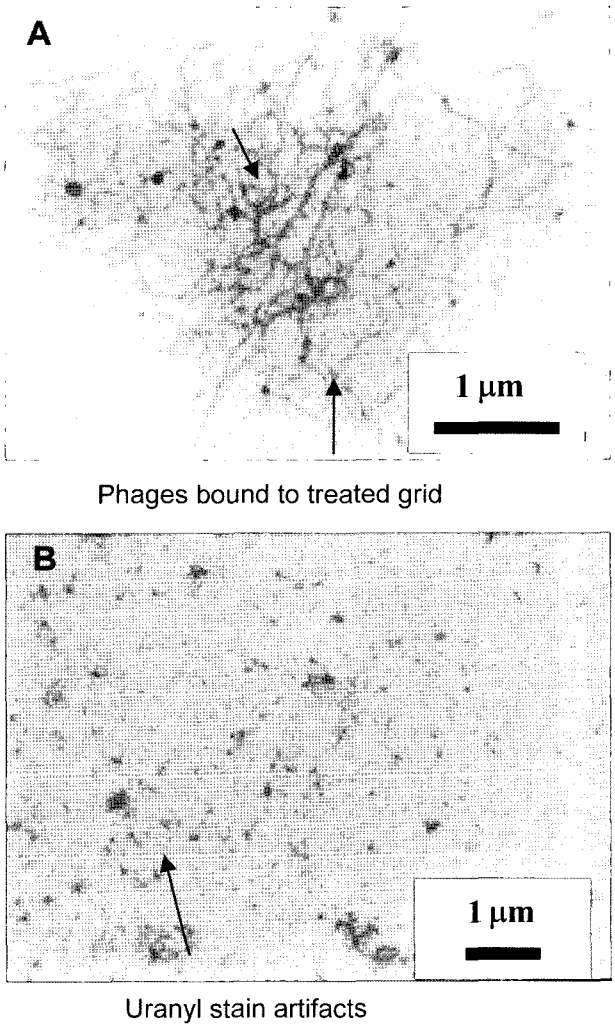

Fig. 1. Determination of dextran binding using transmission electron microscopy. Grids were placed on the drops (10 μl) of SBAE-2R phage antibody (**A**) or Fab 2lox library as a control (**B**), diluted 1:500 with sterile distilled water, for 5 min.

After washing twice with distilled water, grids were placed on the drop (10 μl) of dextran (T10, 500 ppm) dissolved in filtered water, air dried for 5 min, blotted with filter paper to drain excess solution, and then touched to a 20-μl drop containing control Fab 2lox library and rinsed once in filtered water. The grids were exposed to 50 μl of 2% uranyl acetate stain. Arrows indicate phages bound to dextran on grids. Small dots are uranyl staining marks. The marker bar represents 1 μm.

Transmission Electron Microscopy

Maize mosaic virus, in extracts from corn, was quantitated on serologically specific electron microscopy grids, with anti-potato virus Y antiserum diluted from 1:100 to 1:3,200,000. The highest number of the virus was found on grids with an antiserum diluted between 1:100 and 1:1,000 [12]. Serologically specific TEM was also found to be a useful assay for the quantifying dextran-binding phage. Dextran binding of the phage was confirmed by enumeration of phage (SBAE-2R or Fab 2lox library) on TEM grids, which had been coated with anti-dextran mouse monoclonal anti-dextran antibody and dextran (T10, Mw 10^4 daltons). TEM grids were coated with a goat anti-dextran antibody (diluted 1:500). This dilution was found to produce a low background. Phages were quantitated by counting phages per μm^2 TEM images obtained from forty-eight fields on 8 grids. Phages (SBAE-2R) were distributed over the dextran-coated grids with 39±25 phages/μm^2 on the grids (Fig. 1A). Phages were not seen on dextran-coated grids exposed to phage library (Fig. 1B). The small marks on the TEM photographs are uranyl stain artifacts. They are also seen in the negative control grids.

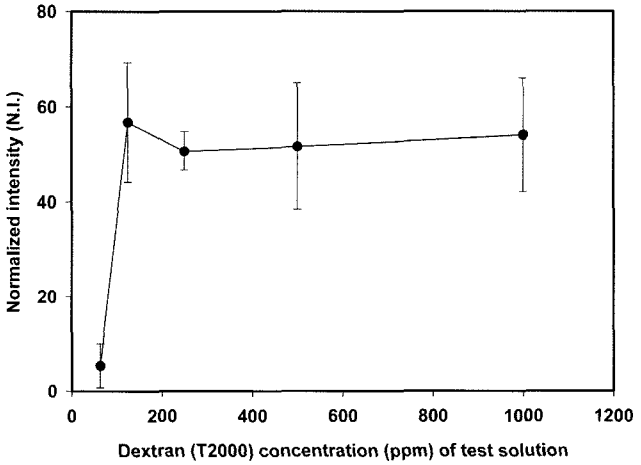

Fig. 2. Detection of dextran. Serial dilutions of dextran (T2000) solution were prepared and the effects of dextran concentration on dextran binding were determined by a paper dip assay using phage (SBAE-2R, 10^5 t.u./ml).

Horseradish peroxidase conjugated anti-M13 antibody and TM Blue substrate were used to detect the presence of phages bound to dextran on the paper, and the developed blue color was scanned by a NucleoVision scanning densitometry system. The error bars represent standard deviations of triplicate experiments (n=6).

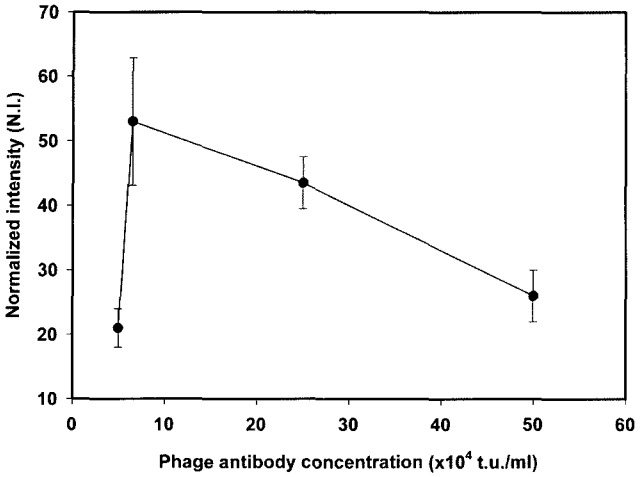

Fig. 3. Effect of phage concentration on dextran bound in a paper dip assay. Phage (SBAE-2R, 5×10^5 t.u./ml) diluted with PBS was used for detection of dextran (T2000, 1,000 ppm). Horse radish peroxidase conjugated anti-M13 antibody and TM Blue substrate were used to detect the presence of phages bound to dextran on the paper, and the developed blue color was scanned by the NucleoVision scanning densitometry system. The error bars represent standard deviations of triplicate experiments (n=6).

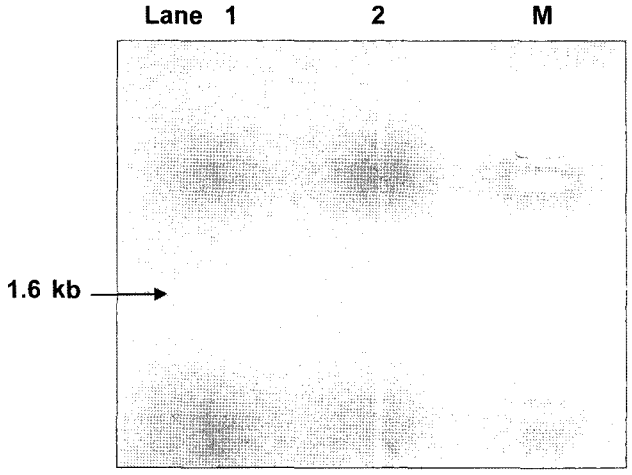

Fig. 4. PCR confirmation of a Fab insert in phage (SBAE-2R). Template DNA was obtained from phage-infected bacterial stocks. Fab DNA amplification was carried out at 94°C, with 5 min for presoaking, followed by 30 cycles of 94°C, 1 min for denaturation at 60°C, 1 min for annealing at 72°C, 1.5 min for extension, and finished by incubation at 72°C for 5 min in a 50-μl PCR reaction mixture using primers Fdpcrback-1 and Fdseq1Forward, 5mM of dNTP, 25 mM of $MgCl_2$, *Taq* polymerase, and *Taq* buffer. Lanes 1 and 2, PCR products of phage (SBAE-2R); M, lambda HindIII size marker.

Effects of Dextran Concentration and Phage Antibody Concentration

A dipstick assay was performed using phage (SBAE -2R). Serial dilutions of dextran (T2000) solution were used to determine the detection limits of phage (SBAE-2R, 2.5×10^5 t.u./ml) in a dipstick assay. The phage produced 54±3 color normalized intensity (N.I.) from 125 ppm to 1,000 ppm of dextran and 5±1 (N.I.) for 63 ppm of dextran (Fig. 2). The phage showed saturation below 125 ppm of dextran, and 63 ppm of test dextran solution produced the lowest dextran detection in this paper dip assay (Fig. 2). This corresponded approximately to the visual detection limit. Phage (SBAE-2R) was serially diluted with PBS, and the effects of phage concentration on detection of dextran (T2000, 1,000 ppm) on the paper dipstick assay were determined. The peak of interest was found at a phage concentration of 6.5×10^4 (Fig. 3). Above this level, N.I. was decreased, possibly because of blinding.

Confirmation of an Fab Insert

The phage clones were tested for the presence of insert DNA within the pIII. *E. coli* TG1 with an Fab insert DNA within the pIII are visually colorless on LB (Luria-Bertani) plates topped with agar containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside, 20 mg/ml; Sigma, St. Louis, MO, U.S.A.) and IPTG (isopropylthio-β-D-galactoside, 200 mg/ml; Sigma, St. Louis, MO, U.S.A.), whereas *E. coli* TG1 without the insert produces blue colonies within 12 to 20 h. Template DNA from phage-infected bacteria after amplification showed a single band of approximately 1.6 kb in 1% agarose gels (Fig. 4). The 1.6-kb bands are considered to be Fab DNA [14]. Polymerase chain reaction and β-galactosidase assay are essential for selection of the desired phages, confirming the presence of Fab inserts after each round of selection.

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