Simple Technique for Separation of Concentrated Immunoglobulin M Fractions by Multi-Sample Gel Chromatography and Its Application to Rubella Serodiagnosis

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A simple technique has been developed with which five serum specimens can be chromatographed at one time and concentrated immunoglobulin M fractions can be obtained without using fraction collectors and ultraviolet monitors. When applied to the detection of rubella immunoglobulin M antibody, this technique gave results comparable to the success gradient centrifugation method.

MATERIALS AND METHODS

The fractionation-concentration technique. (i) Principle. As Fig. 1 shows, the eluate from the chromatography column flows through the peristaltic pump A, and then is divided into fractions by air bubbles intermitently inserted into the tubing by pump B, after which it enters a single hollow fiber whose wall is permeable to water but not to air or proteins. The flow rate of pump A is set to be about one-tenth of that of pump A; thus, proteins in the eluate are concentrated at least 10-fold. The concentrated fractions are collected in vinyl tubing. If a colored substance eluting in the same position as IgM is mixed with the serum sample before chromatography, the fractions containing concentrated IgM are visibly marked. Multiple samples can be chromatographed at the same time by the use of multichannel pumps and corresponding numbers of columns. Air bubbles prevent the mixing of fractions. The concentration ratio would be increased if the volume of an air bubble is increased.

(ii) Apparatus and operation. A five-channel system is shown in Fig. 1. An MHRE/2/DELTA pump (Watson-Marlow, Paltmouth, England) with a 10-channel peristalsis module was used for pumps A and A'. Five channels were used as pump A with silicone tubing with a 1.5-mm bore, and the other five channels were used as pump A' with Auto-Analytical Manifold Tubing (color code: orange yellow; Watson-Marlow). This combination of different tubing gave an approximate ratio of 10:1 in the flow rate between pumps A and A'. For pump B, a 10-channel DELTA C pump (Watson-Marlow) was employed, but half of the channels were used with silicone tubing with a 1-mm bore. The air-insertion line from pump B was connected to the main line with tubing connectors (TC-3; Pharmacia Fine Chemicals, Upplsa, Sweden). The repeating timer was assembled from 60-s and 60-min timers (Tateishi Electric, Kyoto, Japan). Pump B was periodically switched on for 40 s at 30-min intervals. Single hollow fibers (bore, 0.7 mm; length, 135 cm; cutoff molecular weight, 15,000) were purchased from MDA Scientific, Park Ridge, III. Tygon tubing (bore, 1.59 mm, and length, 4 m; Norton, Akron, Ohio) was used for fraction collection.

Gel chromatography. Columns of Ultrogel AcA 34 (1.5 by 90 cm, LKB, Bromma, Sweden) were equilibrated with Veronal-buffered saline, pH 7.3, containing 0.02% NaN3, which was also used as an elution buffer. A 0.6-ml portion of diluted or pretreated serum (see below) was mixed with 0.1 ml of 1% blue dextran 2000 (Pharmacia) and overlaid onto the top of the gel with a syringe with a long needle. The flow rate was set at about 3 ml/h, and chromatography was carried out overnight at room temperature. After completion of the chromatography, the fraction-collecting tubing was removed and connected to another pump. By pumping water into the tubing, the concentrated fractions were transferred to small test tubes.

Determination of immunoglobulin concentrations. The amounts of IgM, IgG, and IgA in the concentrated fractions were determined by single radial immunodiffusion by using Immunoplates (Hyland Laboratories, Los Angeles, Calif.). IgA oligomers were converted to monomers by treatment of the samples with 2-mercaptoethanol; 50 µl of the sample was mixed with 5 µl of 1 M 2-mercaptoethanol, and the mixture
was incubated at 37°C for 1 h.

Pretreatment of serum for detection of rubella hemagglutination-inhibiting IgM antibody. A 0.1-ml portion of serum was treated with heparin-MnCl₂ as previously described (8). The supernatants were further mixed with 0.2 ml of phospholipase C (20 U/ml; P-L Biochemicals, Milwaukee, Wis.) and incubated at 37°C for 2 h. After centrifugation at 900 × g for 20 min, the supernatants were subjected to gel chromatography.

Sucrose density gradient centrifugation. A 0.1-ml portion of serum was mixed with 0.3 ml of Veronal-buffered saline and 0.1 ml of a 50% goose-erythrocyte suspension, and incubated at 4°C for 1 h. After centrifugation at 900 × g for 20 min, the supernatants were centrifuged in sucrose gradients with a Spinco SW 50.1 rotor as previously described (8).

Rubella hemagglutination inhibition test. The
rubella hemagglutination inhibition test was carried out by using a micromethod with goose erythrocytes. Antibody was reacted with antigen at room temperature for 1 h.

RESULTS

Concentrations of immunoglobulins after gel chromatography. Concentrations of three different classes of immunoglobulins in the eluate were assessed. Serum from an adult (IgM, 90 mg/dl; IgG, 1,400 mg/dl; and IgA, 190 mg/dl) was diluted sixfold, and, after mixing with blue dextran, chromatographed on Ultrogel AcA 34. IgM was completely separated from IgG (Fig. 2). The peak concentration of IgM was about one-tenth of the original serum IgM level. IgA eluted between IgM and IgG with a skewed distribution, indicating a heterogeneity of its molecular size.

Application to the detection of rubella IgM hemagglutination-inhibiting antibody. A series of serum samples sequentially taken from a rubella patient (male, 25 years old) were subjected to both gel chromatography and sucrose gradient centrifugation. Hemagglutination inhibiting antibody titers of the first peak (blue dextran position) in the gel filtration were almost the same as those of the 19S peak in the centrifugation (Fig. 3). Hemagglutination inhibiting activity of both peaks disappeared at the same time after the onset of disease. These results indicate that the sensitivity of IgM antibody detection is similar for both techniques.

DISCUSSION

The single hollow-fiber microconcentrator, which played a key role in our technique, was developed by Zeineh et al. (14) who also suggested the use of the fiber in conjunction with column chromatography. We have demonstrated the value of their suggestion and have improved the method by inserting air bubbles for fractionation and by multi-channelization. Air bubbles prevent the trailing of concentrated proteins into the adjacent fractions. Another advantage of air insertion is that the concentration ratio can be increased, without changing the flow rate of pump A', by merely increasing the volume of an air bubble (i.e., by increasing the time interval when pump B is operating).

There have been several reports on the application of gel chromatography for the detection of rubella IgM hemagglutination-inhibiting antibody (3, 6, 10–12). In all cases only single columns were used. In one report (3), to increase the sensitivity of antibody detection, each eluate fraction was concentrated manually; this can be done automatically in our system.

The nonspecific serum inhibitors of rubella hemagglutinins are lipoproteins (2) and elute in the same fractions as IgM. Thus, they must be completely eliminated before chromatography. Since the heparin-MnCl2 method (4, 5) sometimes leaves behind low-titered inhibitors (7, 8), we pretreated the serum with both heparin-MnCl2 and phospholipase C (7, 9). We did not adopt the overnight incubation of the antigen-antibody mixture (1, 10); although this increases the rubella hemagglutination-inhibiting antibody titers, it might also allow such inhibitors, if present, to be detectable.

The void volume fractions on AcA 34 gel chromatography contained IgM as well as oligomeric IgA antibodies. However, the copresence of the oligomeric antibody does not affect the diagnosis of a recent rubella infection since, like IgM antibody, it is also of similar diagnostic value (8, 12).

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LITERATURE CITED


