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Research Note

Effect of a Cross-Linking Reagent on Enzyme-Linked Immunosorbent Assay with *Schistosoma japonicum* Adult Worm and Egg Antigens, and Circulating Anodic Antigen.

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Enzyme-linked immunosorbent assay (ELISA), which is now frequently used as an immunodiagnostic technique, is usually carried out utilizing a polystyrene microtitration tray (Voller *et al.*, 1974). Rotmans and Delwel (1983), and Rotmans and Scheven (1984) have reported that sensitivity in ELISA remarkably increased by using *N*-ethyl-*N'*-(3-dimethyl-aminopropyl)-carbodiimide (EDC), a cross-linking agnet, with *Schistosoma mansoni* adult worms as antigen. However, different results were observed with purified antigens: a large increase in sensitivity was obtained with ovalbumin, transferrin and ferritin, and a small increase with myoglobin, while no change was obtained with bovine IgG, and a decrease in sensitivity was obtained with bovine serum albumin. Timkovich (1977) has reported that EDC catalyzes the formation of intermolecular peptide bonds and polymerizes protein. Therefore, the effect of EDC on ELISA results primarily depends on the chemical structure of each antigen (Rotmans and Scheven, 1984).

In the present study, the effect of antigen cross-linking on ELISA results was assessed by investigating common antigen coating methods using *S. japonicum* egg and adult worm antigens and circulating anodic antigen (CAA)-proteroglycan which exists in schistosome gut epithelial cells (Nash *et al.*, 1977).

Antigens of *S. japonicum* adult worms and eggs collected from rabbits 9 weeks after infection were prepared as reported by Tsuji (1972). Crude CAA was extracted through phenol treatment of adult worm homogenates (Hirata, 1981) and further purified according to the method of Nash *et al.* (1981).

The procedure of ELISA, employing the cross-linking reagent EDC, was performed according to the method of Rotmans and Scheven (1984) with some modifications. The wells of the trays (Dynatech Laboratories, M129A) were coated with 100 μl of 0.025M 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer pH 6.0, containing various concentrations of antigen and 0.1% EDC. The trays were incubated at 37°C for 2 hrs and then at 4°C overnight. The trays were further incubated with 1% bovine serum albumin, rabbit sera, and then peroxidase-conjugated anti-rabbit IgG (1:1000 dilution). The amount of conjugates was determined by incubating the trays with o-phenylenediamine (0.01%) and hydrogen peroxide (0.003%). After 30 minutes the reactions were stopped with 8M sulfuric acid and the intensity of staining reaction was measured at 492 nm using an ELISA autoreader (Hitachi-Corona Co. Ltd.).

In the ELISA, heavily infected (12, 15 and 20 weeks) rabbit sera were used as standard positive sera and 14 normal rabbit sera as negative control sera. In figures, results obtained from a serum of a 20 week-infected rabbit were presented as a representative, because no essential differences were shown among the positive sera. Titers expressed in the present

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study were the reciprocal values of the highest serum dilution which showed higher OD value than 2 fold value of the back ground reaction with antigen alone.

In the study for complex *S. japonicum* adult worm and egg antigens, the effect of antigen cross-linking was assessed by comparison with the other method which used carbonate buffer (0.05M, pH 9.6) in the antigen coating (Nakao *et al.*, 1981). In each experiment, 4 methods of antigen coating were tested: antigen was dissolved in MES buffer or in carbonate buffer each either with or without 1% EDC. Fig. 1 shows the results of ELISA with *S. japonicum* adult worms antigen. Cross-linked antigen gave higher sensitivity than native antigen in either MES or carbonate buffer. Comparison of coating buffers between MES and carbonate buffer revealed higher sensitivity for carbonate buffer, especially at lower concentrations of antigen (3.1 and 0.8 μg/ml). When *S. japonicum* egg antigen was tested, similar results were observed (Fig. 2). With this antigen, the difference between MES and carbonate buffer was more marked.

In the detection of anti-CAA antibody, the method of Kelsoe and Weller (1978) was employed as a standard ELISA. In this method, the wells of plates were pretreated with poly-l-lysine (100 μg/ml) as an intermediate between antigen and plastic. Trays were coated with purified CAA dissolved in phosphate buffered saline (0.01M, pH 7.4) either with or without 1% EDC. Sensitivity in ELISA was determined at a single serum dilution (1:20), because the level of anti-CAA antibody was considerably low in comparison with that of antibody against complex adult worm or egg antigen. Comparison of 4 antigen-coating methods showed that the cross-linked CAA in MES buffer gave strikingly higher OD values than the other antigen-coating methods (Fig. 3). It is noteworthy that the OD value (0.34) shown at 2 μg/ml concentration of CAA dissolved in PBS was comparable to the value at 0.25 μg/ml concentration of CAA in MES

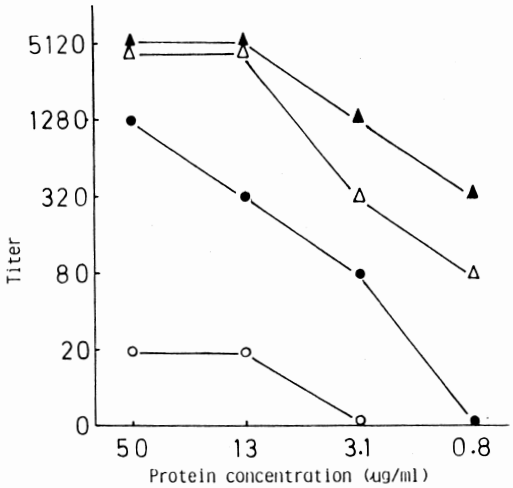

Fig. 1 Comparison of 4 antigen coating methods in the ELISA with *S. japonicum* adult worm antigen.
▲ ; coated with cross-linking antigen in carbonate buffer
△ ; coated with naive antigen in carbonate buffer
● ; coated with cross-linking antigen in MES buffer
○ ; coated with naive antigen in MES buffer

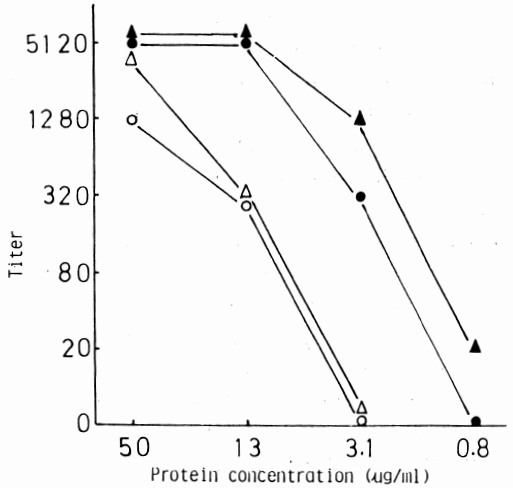

Fig. 2 Comparison of 4 antigen coating methods in the ELISA with *S. japonicum* egg antigen.
▲ ; coated with cross-linking antigen in carbonate buffer
△ ; coated with naive antigen in carbonate buffer
● ; coated with cross-linking antigen in MES buffer
○ ; coated with naive antigen in MES buffer

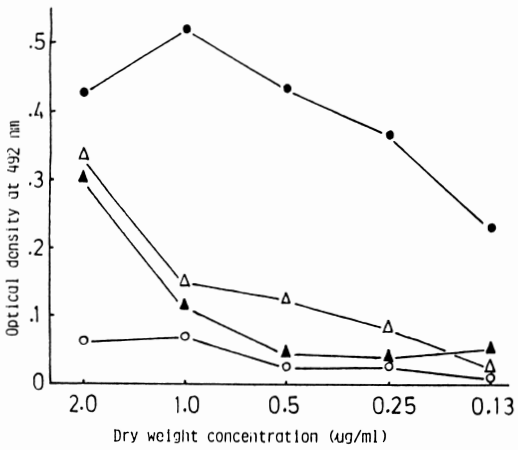

Fig. 3 Comparison of 4 antigen coating methods in the ELISA with circulating anodic antigen.
▲ ; coated with cross-linking antigen in PBS
△ ; coated with naive antigen in PBS
● ; coated with cross-linking antigen in MES buffer
○ ; coated with naive antigen in MES buffer

buffer + EDC. For negative control sera, the reactions were negligible for all antigen coatings.

The present experiments have demonstrated that the antigen cross-linking with EDC is applicable to *S. japonicum* adult worm and egg antigens, and to purified CAA in the ELISA. EDC is usually employed under slightly acidic condition as produced by MES buffer (Timkovich, 1977). A use of carbonate buffer also was found to give satisfactory results for *S. japonicum* adult worm and egg antigen. In the detection of anti-CAA antibody, the method employing antigen cross-linking with EDC presented here made the technique of ELISA more simple compared with that of Kelsoe and Weller (1978). Incubation with poly-l-lysine and subsequent incubation with 5% bovine serum albumin, which is used to block high serum background values caused by non-specific binding to poly-l-lysine, are not necessary in the antigen cross linking method.

The considerable advantage of antigen cross-linking is that sufficient sensitivity is obtained at lower concentrations of antigen. This is particularly useful, whenever materials are poorly available.

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