Short Report

Affinity Chromatography of Hepatitis B Antigen

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SHIRAISHI, H., SUKENO, N., SHIRACHI, R. and ISHIDA, N. Affinity Chromatography of Hepatitis B Antigen. Tohoku J. exp. Med., 1974, 113 (4), 393-394 —— Hepatitis B antigen (HBsAg) in human plasma was purified by affinity chromatography through a column of Sepharose 4B which had been charged with anti-HBs. Eight different elution buffers were examined for better recovery by determining the antigen titers by solid-phase radioimmunoassay (Austria-125). Among the buffers tested, 5M NaI, 1M acetic acid (AcOH) containing 1M NaCl (pH 2.5), and 3.5 M sodium thiocyanate (NaSCN) gave good recoveries and the most preferable result was obtained with 1M AcOH containing 1M NaCl (pH 2.5). —— affinity chromatography; hepatitis B antigen; purification of virus; radioimmunoassay

HBsAg (cf. Reference 1) is a lipoprotein with a molecular weight of $3 \times 10^6$ daltons and associated with hepatitis B virus infection (Dreesman et al. 1972). The purification of HBsAg has been hitherto performed by various combinations of time-consuming centrifugation steps (Sukeno et al. 1972; Vyas et al. 1972) and resulted in unsatisfactory recovery. In the present communication we examined the conditions for purification of HBsAg by anti-HBs (cf. Reference 1) affinity chromatography as a review of the literature revealed that various elution buffers have been used by different researchers (Tripathis and Horst 1971; Grabow and Prozesky 1973) and recovery of HBsAg in each case was not clear.

Twenty ml of Sepharose 4B (Pharmacia Fine Chemicals, Inc., Sweden) was suspended in 20 ml of distilled water and to this was added 20 ml of aqueous solution of 5 g of cyanogen bromide. pH of the suspension was adjusted to 11.0-11.5 by 4N NaOH until the pH remained constant for 8 to 15 minutes. The suspension was cooled down to 20°C and was rapidly filtered through Buchner funnel. Then the Sepharose 4B was washed repeatedly with 500 ml of ice cold 0.5M NaHCO$_3$ containing 0.5M NaCl (pH 9.0) within 5 minutes. Thus activated Sepharose 4B was suspended in 20 ml of 0.5 M NaHCO$_3$ containing 0.5 M NaCl (pH 9.0) and 5 ml of horse anti-HBs γ-globulin solution (20 mg/ml). The suspension was stirred gently overnight at 4°C. Anti-HBs-labeled Sepharose 4B washed several times with the above buffer. Then 1M ethanolamine, pH 8.0, was added to mask the remaining active residues. After 1 hr, the Sepharose 4B filtered was washed with 0.1 M acetic acid containing 0.1M NaCl (pH 4.0) and 0.1M borate containing 0.1M NaCl (pH 8.0) alternatively 3 times, then with distilled water and finally with 0.05M Tris-HCl containing 0.1M NaCl (pH 7.2). The anti-HBs-labeled Sepharose 4B thus prepared was packed in parallel eight columns and to each column an equal amount of serum containing HBsAg was applied. The column was washed with Tris buffer (pH 7.2), until unadsorbed proteins were completely eluted out. HBsAg adsorbed to the columns was eluted with 8 different elution buffers. Each eluate was dialyzed, concentrated, and the titer was measured (Table 1).

Received for publication, July 8, 1974.
It is evident in the table that the best buffer with respect to the eluting efficiency of HBsAg was 5M NaI (Grabow and Prozesky 1973) (RIA titer 211 and IES titer 22) and the second best was 1M AcOH containing 1M NaCl (pH 2.5), or 3.5M NaSCN (RAI titer 211 and IES titer 22). The glycine-HCl (pH 2.5) which has been used most frequently was less efficient than the above three and was comparable to the elution with 2.5M MgCl₂ or 5M KI. On the other hand, the amounts of human serum components in each eluate as determined by IES titration were least in 1M AcOH containing 1M NaCl (pH 2.5) or glycine-HCl (pH 2.5) (IES titer 22). 5M NaI and 3.5M NaSCN which gave good recoveries of HBsAg also contained a large amount of human serum components as contaminants (IES titer 22 and 22). Also, when the eluates were concentrated against carbowax-20000, precipitation of considerable amounts of protein was observed and a relative amount of protein in each elution system was shown in the right column of the table. In the eluates with 5M NaI and 3.5M NaSCN more abundant precipitates were formed than with other buffers. Considering that NaI or NaSCN must have some influence on the solubility of proteins and that the acetate buffer-eluate contained least amount of human serum components other than HBsAg, we conclude that the most preferable buffer for eluting HBsAg from the affinity column is 1M AcOH containing 1M NaCl (pH 2.5). That human serum components as contaminants can be minimized by choosing elution buffer is very important to proceed to next purification steps.

References