# PURIFICATION OF ANTIBODIES TO PROTEIN HORMONES BY AFFINITY CHROMATOGRAPHY ON DIVINYLSULFONYL SEPHAROSE[†]

M. R. Sairam, W. Craig Clarke,[*]

David Chung, Jerker Porath[§], and Choh Hao Li[¶]

Hormone Research Laboratory
University of California
San Francisco, California 94143

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SUMMARY: Antibodies to human growth hormone and ovine interstitial cell stimulating hormone have been purified from rabbit antisera by affinity chromatography using a newly developed divinylsulfonyl activated agarose. Elution of the antibodies was accomplished by neutral solutions containing chaotropic ions.

The technique of biospecific affinity chromatography (1, 2) has been employed in the isolation of enzymes (3), antibodies (3), and hormones (4-6). Derivatives of agarose activated by cyanogen bromide (7) have been widely employed as a solid support for immobilizing ligands (1, 2). In this communication we wish to describe the use of divinylsulfonyl activated cross linked sepharose (DVS-sepharose 6B)[‡], a new solid support (8) for the single step isolation of antibodies to ovine ICSH and HGH.

## EXPERIMENTAL AND RESULTS

The procedure for the immobilization of ovine ICSH and HGH on

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[†] Supported in part by USPHS Grant AM-6097 and the American Cancer Society.
[*] Medical Research Council of Canada Postdoctoral Fellow, 1972-1974.
[§] Visiting Research Professor, 1974. Permanent address: Institute of Biochemistry, University of Uppsala, Sweden.
[¶] To whom correspondence should be sent.
[‡] Abbreviations: DVS, divinylsulfonyl; ICSH, interstitial cell stimulating hormone; HGH, human pituitary growth hormone; NaTFA, sodium trifluoroacetate; NaTCA, sodium trichloroacetate.

DVS-sepharose was as follows: 10 ml of the activated gel suspension (DVS-sepharose 6B, Lot No. 731176) was thoroughly washed with 0.25 M $NaHCO_3$; 20 mg of HGH (9) dissolved in 10 ml of the same solution was added to the washed gel. The mixture was gently stirred overnight at 23° C. To ensure complete blocking of all the reactive groups on the DVS-sepharose, 1 g of glycine was added, and the mixture was stirred for two hours. The gel was then extensively washed on a coarse sintered glass funnel with 0.1 M Tris buffer of pH 7.6 containing 0.3 M NaCl and 0.02% azide. The procedure for coupling ovine ICSH (10) to the DVS-sepharose was as described for HGH except that 10 mg of the hormone was used to couple to 6 ml of the activated gel. Amino acid analysis of the acetone dried gel estimated 30% coupling in the case of HGH, while optical density of the supernatant obtained after terminating the reaction indicated the coupling of ovine ICSH was 85-90%. The immobilized hormone gels were stored at 4° C in the same buffer until use.

Potent rabbit antisera against the two hormones were prepared according to published procedures (11). The antisera were passed through the immobilized hormone column (2-10 ml) at a very slow rate to allow sufficient time for the binding of the antibody to the hormone. The serum proteins were eluted out as a large peak with the starting buffer as shown in Figures 1 and 2. Stepwise elution was performed successively with 1 M NaTFA, 2 M NaTFA, 1 M NaTCA, and 2 M NaTCA, all at pH 7.0 (Figures 1 and 2). After equilibration with starting buffer, the affinity column was ready for another chromatography run. Aliquots of the same antiserum gave reproducibly results in as many as six separate experiments. However, the elution pattern did vary among sera obtained from different rabbits immunized with the same preparation of antigen.

The immunodiffusion technique of Ouchterlony (12) was employed to

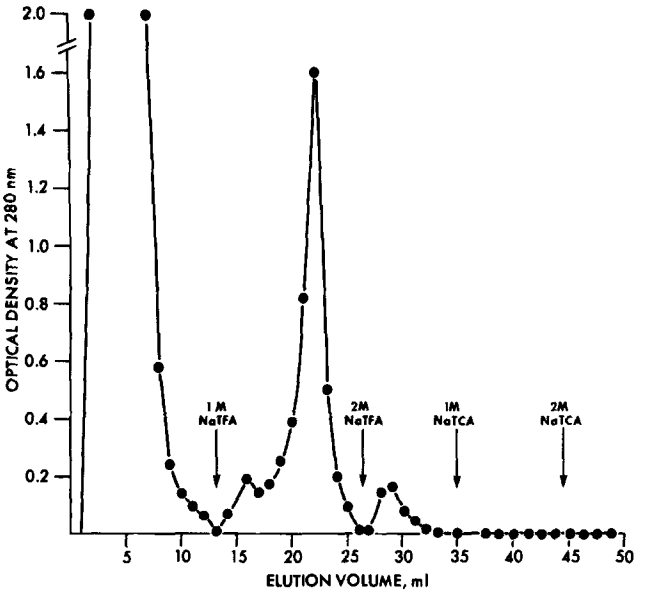


Figure 1: Rabbit antiserum to ovine ICSH (5 ml) passed through a 2 ml column of DVS-sepharose-6B-ICSH. Stepwise elutions were performed as indicated with various solutions of pH 7.0. Flow rate, 2.5 ml/hour; 1ml/tube.

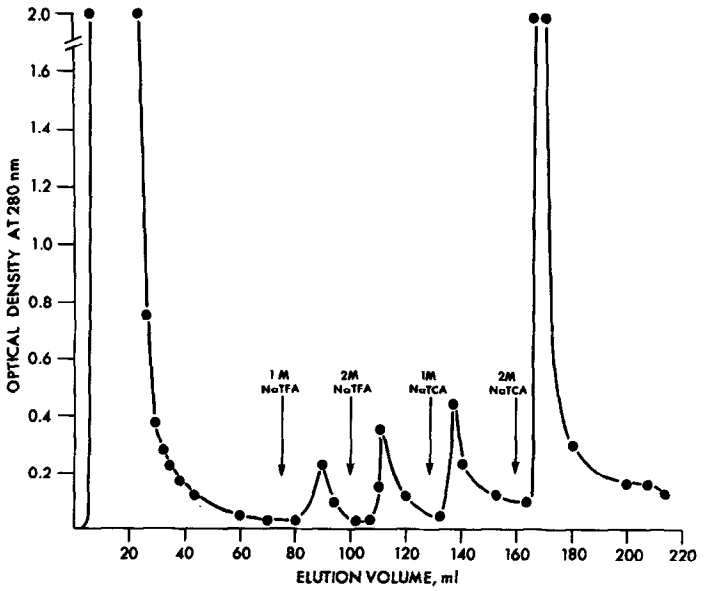


Figure 2: Rabbit antiserum to HGH (5 ml) on a 10 ml column of DVS-sepharose-6B-HGH. Stepwise elution as Figure 1. Flow rate, 12 ml/hour; 1 ml/tube.

screen the eluates for antibody activity. Antibody to ICSH was eluted with 1 M NaTFA (Figure 1) whereas antibody to HGH was eluted with 2 M NaTCA (Figure 2). The eluted HGH antibody was promptly diluted with the starting buffer to minimize exposure to 2 M NaTCA. Pooled fractions from several runs were dialyzed against several changes of 0.025 M $NaHCO_3$ at 4° C and concentrated by evaporation at 23° C in a stream of air over the dialysis bag. The eluted ICSH and HGH antibody fractions contained $I_gG$ but not $I_gA$ as shown in agar diffusion tests (12) with monospecific goat antisera (Miles Laboratories). The recovery of antibodies to both hormones was 20-25% as estimated by microcomplement fixation (13).

## DISCUSSION

In affinity chromatography, the dissociation of antigen-antibody complexes on column has been accomplished using highly acidic solutions or denaturants (guanidine hydrochloride or urea) or a combination of both. Such drastic conditions frequently lead to undesirable damage of many proteins. It is evident from the above results that we were able to dissociate the complex using the chaotropic ions trifluoroacetate and trichloroacetate in neutral solutions (8).

The application of gradient elution with chaotropic agents might be more effective than stepwise elution, however, several notes of caution should be pointed out. The elution pattern of the antibody fraction may vary not only with the hormone (antigen), but also with the batch of antiserum employed. Repeated use of an immobilized hormone column could also result in altered elution patterns. It has been noted in this study that some antibodies were denatured by extensive exposure to 2 M NaTCA as indicated by turbidity of the eluate. Even partial denaturation renders them unsuitable for complement

fixation studies. In view of these potential variations, we suggest the chromatographic runs be standardized with each batch of the immobilized hormone and a series of eluents be employed for every batch of antiserum in order that the antobody be obtained under the mildest possible conditions to be useful for subsequent applications. ‡

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‡ The isolated antibody to ICSH has been useful in the isolation of biologically active ICSH from individual sheep pituitaries by affinity chromatography on DVS-sepharose 6B as described in this paper.