ISOLATION OF ANTIBODIES TO PROTEIN HORMONES BY BIOAFFINITY CHROMATOGRAPHY ON DIVINYLSULFONYL SEPHAROSE *

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SUMMARY: Antibodies to ovine and human interstitial cell stimulating hormone, the β subunit and rat pituitary prolactin were isolated by affinity chromatography on divinylsulfonyl sepharose 4B. Highly purified antibodies in good yield were eluted in all cases with 0.5 or 1 M ammonium hydroxide at 4°C. The eluted γ-globulin fraction retained good antibody activity.

The selective isolation and purification of biologically active macromolecules by the recent technique of biospecific affinity chromatography exploits their unique biological property to bind to ligands in a specific and reversible manner (1,2). This technique has found application in the isolation of enzymes (2) antibodies (2,3) and hormones (4,6). Most of these investigations have employed derivatives of agarose activated by cyanogen bromide (7,8) as solid support for the preparation of immobilized ligands such as proteins. We recently described (3) the use of divinylsulfonyl sepharose (DVS-sepharose), a new solid support (9) for the isolation of antibodies. This communication describes conditions for the preparation of antibodies in high yield.

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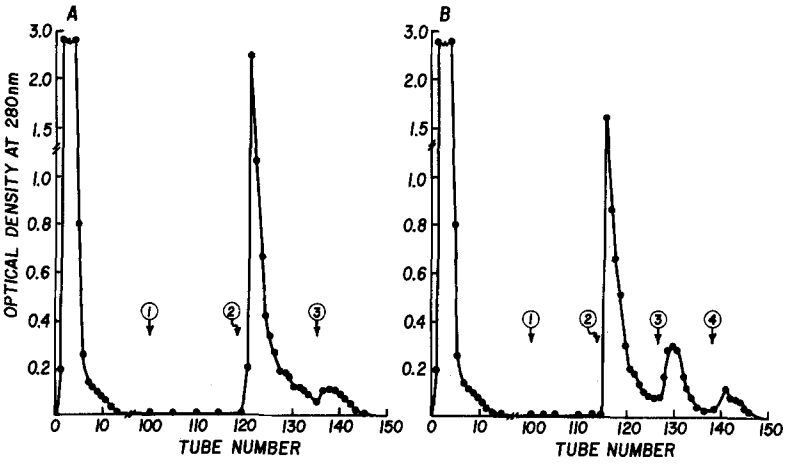
Abbreviations: DVS - divinylsulfonyl; ICSH - interstitial cell stimulating hormone; HCG - human chorionic gonadotropin; rPr - rat prolactin.

EXPERIMENTAL AND RESULTS

Antigens: Ovine ICSH was prepared essentially as described earlier (10) and its subunits were obtained by the salt precipitation method (11). Human pituitary ICSH (12) was prepared from frozen glands. HCG was purified (13) from a semi pure fraction obtained from Organon Laboratories. Rat pituitary prolactin (I-1 and B1) was supplied by the NIH - hormone distribution program. Antisera to ovine ICSH, ovine ICSH-β, human ICSH and rat prolactin were prepared in male rabbits according to published procedure (14).

Three hormones, ovine ICSH, HCG and rat prolactin were coupled to DVS-sepharose. The activated DVS-Sepharose -4B (9) which had been stored at $4^{o}C$ was thoroughly washed with 0.25 M $NaHCO_3$ at pH 9.0 containing 0.02% sodium azide. Ovine ICSH, 10 mg., dissolved in 6 ml of 0.25 M $NaHCO_3$ pH 9.0 was mixed with 5 ml of gel suspension and shaken gently for 6 hours at $23^{o}C$ on an end-over-end shaker. The extent of coupling was determined by optical density measurements at 280 nm to be approximately 75-80%. Addition of 1 gm of glycine along with fresh 0.25 M $NaHCO_3$ pH 9.0 terminated the coupling and served to block the remaining reactive groups. The gel was then extensively washed with 0.1 M Tris-HCl pH 7.6 containing 0.3 M NaCl and kept at $4^{o}C$ until use. In the same manner 10 mg HCG and 5 mg rat prolactin were coupled. The coupling efficiency was approximately the same as for ICSH.

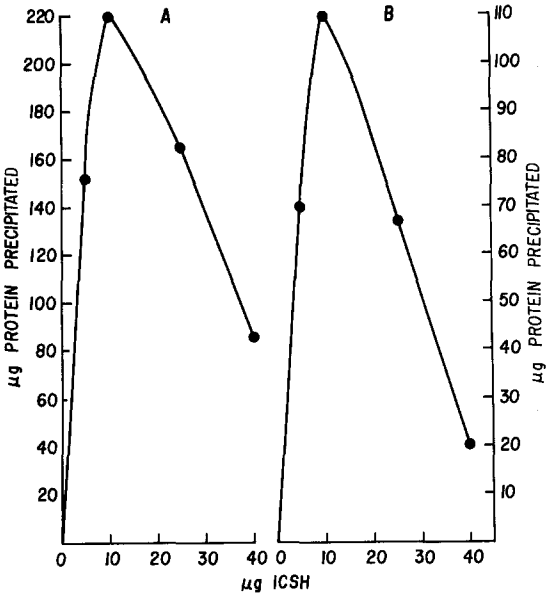
Two ml of antiserum to ovine ICSH was passed through the immobilized hormone contained in small Bio-Rad columns which are very well suited for this purpose. The antiserum was circulated through the column at $23^{o}C$ by means of a peristaltic pump for 3 hours to ensure complete reaction of the antigen-antibody. The serum proteins were eluted as an unadsorbed peak (fig. 1A) and the column was thoroughly washed with the 0.1 M Tris - HCl-NaCl buffer pH 7.6 at room temperature. This was then followed by a wash at $4^{o}C$ with 0.05 M NH_4HCO_3



1. Fractionation of 2 ml of rabbit antiserum (8 mg antibody) to ovine ICSH on a 1.5 ml column of DVS-sepharose-4B-ICSH. Stepwise elution of 4°C as indicated by arrows. A. 1-0.05 M NH_4HCO_3, 2-0.5 M NH_4OH, 3-1 M NH_4OH. B. 1-0.05 M NH_4HCO_3, 2-0.1 M NH_4OH, 3-0.5 M NH_4OH, 4-1 M NH_4OH. Flow rate 20 ml/hr., 1.2 ml/tube. Total yield of γ-globulin was 6.4 mg (80% recovery).

which served to remove all the non-volatile salts from the column. Elution of the γ-globulin was achieved with either 0.5 M or 1 M NH_4OH at 4°C. Elution was normally completed in about 90 minutes and the γ-globulin was recovered by direct lyophilization. The results were reproducible with ovine ICSH using 3 different rabbit antisera and antiserum obtained at different bleedings from the same rabbit.

The lyophilized γ-globulin fractions were tested for antibody activity by the agar gel double diffusion method (15). Initial experiments indicated that the majority of the antibody was eluted with 0.5 M NH_4OH while 1 M elution consistently yielded a small amount of γ-globulin, presumably of higher binding affinity. Quantitative precipitin (14) (fig. 2) tests indicated that



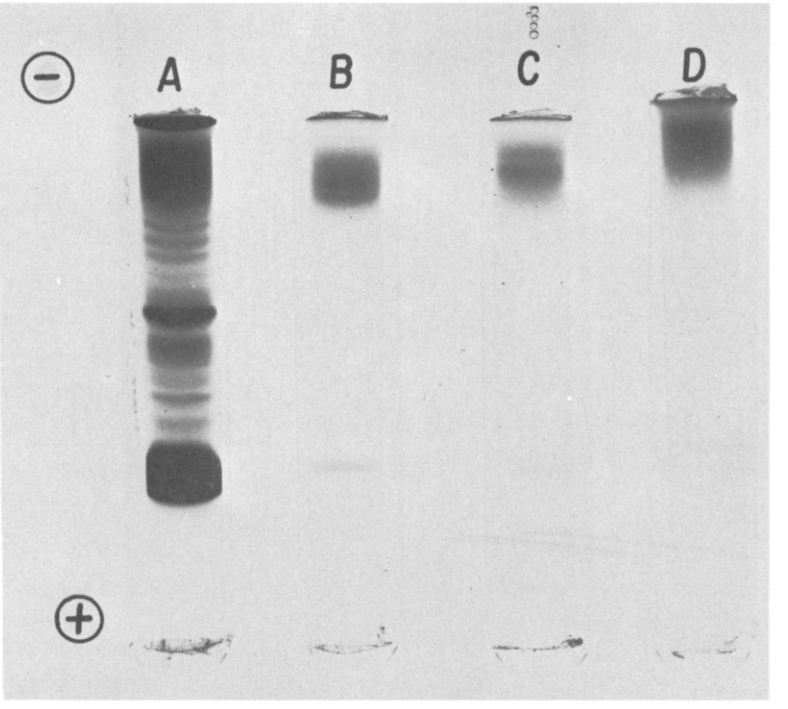
2. Quantitative precipitin tests with ovine ICSH antiserum (A) and isolated γ-globulin (B). Total volume was 0.2 ml. A - 50 µl of antiserum. B - 150 µg of γ-globulin (some insoluble material was removed by centrifugation).

about 65-80% of antibody in 2 ml of the ovine ICSH antiserum was recovered on a weight basis.

Disc electrophoresis (fig. 3) indicated that the γ-globulin obtained was highly purified. We have earlier shown that the eluted antibody fractions are of the IgG type (3). Similar results were obtained when (1) ovine ICSH-β antiserum was passed through the DVS-S-ICSH column; (2) human ICSH antiserum was used with the DVS-S-HCG column and (3) rat prolactin antiserum was filtered through the immobilized rat prolactin column.

DISCUSSION

In bioaffinity chromatography it is essential to use efficient but milder conditions to bring about the dissociation of the antigen-antibody complexes. We recently reported that chaotropic ions such as trifluoroacetate and trich-



3. Disc electrophoretic patterns of isolated γ-globulin. A - ovine ICSH antiserum (5 µl). B - ovine anti-ICSH-γ-globulin 100 µg. C - ovine anti-ICSH-β, γ-globulin 100 µg. D - rabbit γ-globulin (Miles Labs Inc.) 100 µg. pH 8.3, 7% acrylamide 3 mA/tube/hr. Amido black stain.

loroacetate in neutral solutions (9) could be used as substitutes for the drastic conditions normally employed in the past. Despite their ability to elute the antibodies (3), the use of chaotropic agents was not free from problems. We were concerned with the relatively poor recovery and partial denaturation of the eluted antibody by rather extensive exposure to sodium trifluoroacetate or trichloracetate. It is evident from the results presented here that the use of NH_4OH (0.5 or 1 M) overcomes these problems and offers additional advantages. Besides high recovery, the purified γ-globulin can be obtained in a lyophilized form. Its solubility properties are good though a small proportion of it remains insoluble in the aqueous solutions employed.

The immobilized ovine ICSH column can be repeatedly used. We have run as many as twenty-five separate experiments (passing 2 ml antiserum each time) on a single 1.5 ml column with highly reproducible results. The specificity of the column is indicated by the lack of protein in 1 M NH_4OH eluates when normal rabbit serum or a non-cross-reacting antiserum was passed through the column after the 10th or 15th run. The rather high alkaline pH* does not seem to affect the performance of the column. The 0.5/1 M NH_4OH elutes the γ-globulin irrespective of the hormone (antigen) or the batch of the antiserum employed. In these studies the immobilized columns were also used to purify cross-reacting antibodies - e.g. ovine ICSH column to purify ovine ICSH-β anti-γ-globulin and HCG column to isolate anti-γ-globulin to human pituitary ICSH.

It is also possible to fractionate anti-γ-globulin of presumably different affinities by using more dilute solutions of NH_4OH (fig. 1B) such as 0.1 M or 0.25 M. Even 0.01 M NH_4OH can displace a significant proportion of the total γ-globulin. The utility of the isolated antibodies in various experiments is currently being evaluated.

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REFERENCES

1. Cuatrecasas, P. and Anfinsen, C.B. (1970) Ann. Rev. Biochem. 40, 259-278.
2. Affinity Techniques: Enzyme Purification Part B: "Methods in Enzymology". (1974) Eds. W.B. Jakoby and M. Wilchek. Acad, Press, New York.

* It is unlikely that the use of 1 M NH_4OH at 4^oC is causing denaturation of γ-globulin. The same treatment of ovine ICSH which has a quaternary structure does not result in dissociation. In some experiments we have used 0.1 M acetic acid at 4^oC for elution of the γ-globulin but this is not recommended as this has been found to result in significant dissociation of ovine ICSH (unpublished results).

3. Sairam, M.R., Clarke, W.C., Chung, D., Porath, J. and Li, C.H. (1974) Biochem. Biophys. Res. Commun. 61, 355-359.

4. Guyda, H. and Friesen, H.G. (1971) Biochem. Biophys. Res. Commun. 42, 1068-1075.

5. Hwang, P., Murray, J.B., Jacobs, J.W., Niall, H.D. and Friesen, H.G. (1974) Biochemistry 13, 2354-2358.

6. Gospodarowicz, D. (1972) J. Biol. Chem. 247, 6491-6498.

7. Axen, R., Porath, J. and Ernback, S. (1967) Nature 214, 1302-1304.

8. Porath, J., Axen, R. and Ernback, S. (1967) Nature 215, 1491-1492.

9. Porath, J. in ref. 2, p. 13-30.

10. Papkoff, H., Gospodarowicz, D., Candiotti, A. and Li, C.H. (1965) Arch. Biochem. Biophys., 111, 431-438.

11. Sairam, M.R. and Li, C.H. (1974) Arch. Biochem. Biophys. 165, 709-714.

12. Sairam, M.R. and Li, C.H. Biochim- Biophys. Acta (in press).

13. Bahl, O.P. J. Biol. Chem. (1969) 244, 567-574

14. Moudgal, N.R. and Li, C.H. (1961) Arch. Biochem. Biophys. 95, 93-98.

15. Ouchterlony, O. (1953) Acta Pathol. Microbiol. Scand. 32, 231-240.