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# Insoluble antibody column for isolation and quantitative determination of gibberellins

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A column that specifically binds gibberellins was prepared by covalent binding of anti-GA antibodies to Sepharose. By using such an immunoadsorbent column, radioimmunoassay for quantitative determination of gibberellins was developed. Using this method, diffusible GA from apices of etiolated pea seedlings was isolated in one step and determined quantitatively.

During the last decade, various modifications of immunological methods have been applied to the detection and assay of proteins, polypeptides and animal hormones. These methods are in general based upon the ability of an antibody to specifically bind its antigen labeled with a radioactive isotope (*1*) or otherwise modified (*2*) and the competitive inhibition of this reaction by the unlabeled antigen. The chemical coupling of proteins to agarose beads has been described (*3*) and the use of antibody-agarose conjugates for affinity chromatography has been reported (*4*). The production and use of antibodies with specificity toward gibberellic acid, for determination of the hormone, have been reported recently (*5*, *6*). Anti-gibberellic acid ($GA_3$) antibodies were shown to react similarly with $GA_1$, $GA_3$ and $GA_5$. We describe here the preparation of an anti-$GA_3$-Sepharose column and our attempts to use it for affinity chromatography and radioimmunoassay of gibberellins.

## Materials and methods

*Purification of anti-GA-antibodies*

Specific antibodies to GA were purified using an immunoadsorbent prepared by conjugating G-BSA ($GA_3$ conjugated to bovine serum albumin) to Sepharose, which had been previously activated with cyanogen bromide (*3*) by suspending 20 g (wet weight) of Sepharose (4 B) in 75 ml water, then adding 1.7 g of cyanogen bromide. The pH was raised immediately to 11.0 and kept there for 8 min by adding 2 N NaOH. This activation reaction was terminated by filtering the suspension and washing it several times with cold water and 0.1 M $NaHCO_3$. The washed activated Sepharose was added to 40 ml of 0.1 M $NaHCO_3$ containing 150 mg of G-BSA. Coupling was allowed to proceed for 15 hr with gentle stirring at 4°C, after which the conjugated Sepharose was filtered and washed with cold

phosphate-buffered saline (PBS, i.e., 0.01 M phosphate buffer pH 7.4, 0.15 M NaCl), until the absorbance at 280 nm of the washing fluids was less than 0.02. The washed immunoadsorbent was incubated for 1 hr in 0.5 M acetic acid to simulate the conditions of antibody elution, as will be described below. After acid treatment, the immunoadsorbent was washed several times with cold PBS.

*Purification of anti-GA antibodies using* G-BSA-Sepharose as an immunoadsorbent was performed as follows: 100 ml of anti-gibberellyl-hemocyanin (G-Hc) serum (*6*) was suspended with the immunoadsorbent with gentle stirring for 1 hr. The suspension was filtered. The residual serum after absorption failed to precipitate with G-BSA or G-Hc. The immunoadsorbent was washed with cold PBS until the absorbance (280 nm) was less than 0.02. Dissociation and elution of the antibodies from the immunoadsorbent were performed with 0.5 M acetic acid. The eluted protein solution was dialyzed against several changes of cold PBS, and the antibody solutions thus obtained were concentrated by pressure dialysis.

*Insoluble antibody column*

Anti-gibberellic acid antibodies, purified as described above, were used for the preparation of a column of anti-GA-Sepharose, in the same way as G-BSA was conjugated to Sepharose (above). Each gram of the Sepharose contained about 15 mg bound antibodies. The antibody-Sepharose adsorbent was packed into small

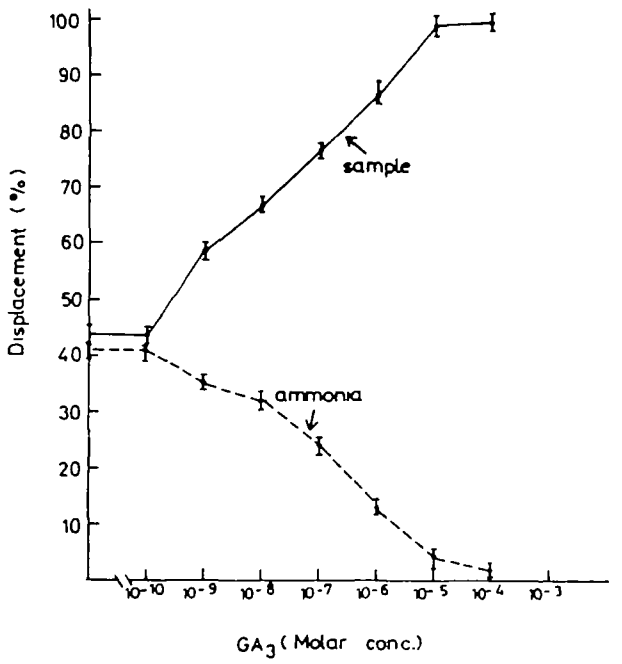


Fig. 1. *Displacement of tritium-labeled $GA_3$ by unlabeled $GA_3$.* The radioactive $GA_3$ was applied first and bound to the column then various amounts of unlabeled $GA_3$ were applied. The radioactivity (CPM) of the samples eluted with $GA_3$ and with ammonia was determined. Percent displacement and standard error of the mean were calculated.