

Addition of alcohol up to 5 % to the dialyzed solution usually precipitates a black colored inactive protein. The solution is then again dialyzed, this time against a pyrophosphate buffer, pH 7.8, until most alcohol is removed. At this pH more inactive protein could be precipitated by adding as much as 20 % alcohol, and the alcohol is subsequently removed by dialysis against acetate buffer 0.1 M, pH 5. At this step the purity is about 10 000–15 000. From now on only ammonium sulphate fractionation was used. Fractionation at pH 4.2 and at pH 5 yields a product with 20 000–30 000 Units per mg. Careful fractionation at pH 6 then raises the purity of the enzyme to about 50 000–60 000 with a fairly good yield.

The pH is adjusted to 7.8 and the enzyme comes down first on addition of ammonium sulphate. Twice crystallized the activity was about $1.3-1.7 \times 10^5$ Units per mg, A_{290} for 1 mg per ml is 1.4. When the crystals are dissolved in water and dialyzed against distilled water the enzyme crystallizes, see Fig. 1.



Fig. 1. Crystalline β -glucuronidase ($\times 400$).

Stability. The enzyme is stable from about pH 3.7 to 8. It can be frozen and thawed several times without loss. Dialysis slowly inactivates the enzyme. In ammonium sulphate solutions the enzyme has been kept for months at zero degrees without loss of activity.

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The Influence of Dextran on the Precipitin Reaction

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Although various polymers, such as dextran, gelatin, polyvinylalcohol, and polyvinylpyrrolidone have been used to increase the sensitivity of immunoreactions *in vitro* (cf. e.g. Ref.¹) the role of the polymers in the reactions has not been elucidated. Recently Laurent^{2,3} discussed the steric interaction between polysaccharides and proteins. He showed that this interaction decreases the solubility of the proteins; the larger the protein, the larger the effect. Such a steric interaction could account for the effect of polymers in immunoreactions. The interaction should then be most pronounced in the region of antigen excess, where it is believed that high-molecular weight antigen-antibody complexes exist in solution.⁴

Stahman and Matthews⁵ showed that small amounts of polylysine increased the

rate of precipitate formation between tobacco mosaic virus and its antibody. Furthermore, in the presence of polylysine, precipitation was observed even in the region of antigen excess where precipitation does not ordinarily occur. Further evidence for decreased solubility of antigen-antibody complexes in the presence of polymers has already been given by Albertsson and Philipsson,⁸ who, in studies of the partition behavior of antigen-antibody complexes in two-phase polymer systems, observed precipitates at the boundary between the phases even in the antigen excess region.

This communication deals with the effect of dextran on the precipitin reaction.

Human serum albumin (Lot No. RbH 23) was kindly supplied by Mr. H. Björling, AB Kabi, Stockholm. It was freed from high and low molecular weight contaminants by gel filtration on Sephadex G-200⁷ and was labelled with ¹²⁵I by the method of McFarlane⁸ (2.8 μ C per mg protein). The γ -globulin fraction of rabbit anti-albumin sera was prepared by adsorbing other proteins on DEAE-Sephadex at an ionic strength of 0.05, pH 7.4⁹ and subsequent gel filtration of the unadsorbed material on Sephadex G-200. Dextran with a weight-average molecular weight of 80 000 was kindly supplied by Dr. K. Granath, AB Pharmacia, Uppsala.

The precipitin reaction¹⁰ was performed by mixing 4 mg of γ -globulin, 0.01–0.6 mg of albumin and 0, 4, 8, or 16 mg of dextran in 0.4 ml of 0.05 M phosphate buffer, pH 7.3, containing 0.1 M sodium chloride. The mixtures were allowed to stand at room temperature for half an hour and then at +4°C for 60 h before they were centrifuged. The supernatants were decanted off and the precipitates were washed with 0.4 ml of the corresponding dextran solutions. Each of the precipitates was then dissolved in 1.0 ml of 0.2 M sodium hydroxide. The radioactivities of the supernatants, precipitates and the washing solutions were assayed in a scintillation counter.

The results are shown in Fig. 1, where the radioactivity in the precipitates has been plotted against the amount of albumin in the reaction mixture. In the region of antibody excess (albumin < 150 μ g), nearly complete precipitation of radioactive material was observed in the four experimental series. In the region of antigen excess (albumin > 200 μ g), the addition of dextran had a marked effect on the precipitation

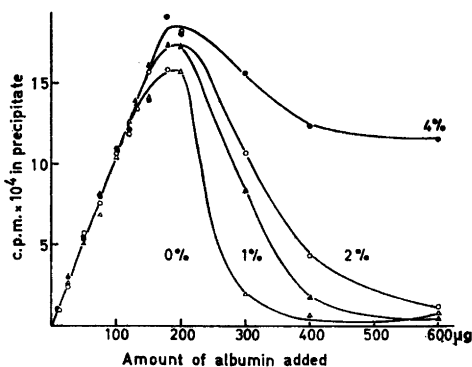


Fig. 1. Quantitative precipitin curves obtained in the presence of various concentrations of dextran. To a constant amount of rabbit antibodies against human serum albumin were added varying amounts of ¹²⁵I-labelled serum albumin. The amount of antigen in the precipitate was measured in a scintillation counter.

curves; (e.g., with a three-fold excess of albumin, hardly any precipitate was formed in the absence of dextran, whereas the precipitate obtained in the presence of 4% dextran was about 65% of that found at equivalence.)

These investigations will be described in detail in a later publication.

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