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Short Communications

Precipitation of Arabic Acid and some Seed Polysaccharides by Glycosylphenylazo Dyes

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The use of 1,3,5-tri-(*p*-α-L-fucosyloxyphenylazo)-2,4,6-trihydroxybenzene for precipitation of an L-fucose-binding protein from the seed of *Lotus tetragonolobus* (Yariv, Kalb & Katchalski, 1967) revealed that some polysaccharide components present in aqueous extracts of various seeds are precipitated with this dye and with the related glucoside dye 1,3,5-tri-(*p*-β-D-glucosyloxyphenylazo) - 2,4,6 - trihydroxybenzene (Yariv, Rapport & Graf, 1962). We observed that arabic acid is also precipitated with the glucoside dye. In this communication we describe the precipitation reaction and a procedure for the isolation of some seed polysaccharides based on this reaction.

Precipitation of arabic acid (Pfanstiehl Laboratories Inc., Waukegan, Ill., U.S.A.) by the glucoside dye was effected at room temperature in 0·1 M-sodium phosphate buffer, pH 6·8, in the range of 5–100 μg. of polysaccharide/ml. A critical ratio of dye to arabic acid is necessary for precipitation. A plot of precipitated dye versus added dye is concave upwards at low dye concentration and becomes concave downwards at higher concentration, eventually reaching an asymptote. The asymptotic value of precipitated dye is proportional to the amount of polysaccharide. In an experiment, glucoside dye was added to each of a series of conical test tubes containing 40 μg. of arabic acid in 2 ml. After 1 hr. the mixtures were centrifuged and the concentration of dye in the supernatants was measured. Precipitates were observed in all mixtures containing more than 12 μg. of added dye. The saturation value of 41 μg. of bound dye was reached with 100 μg. of added dye and persisted up to 400 μg. of added dye. With a 1 : 1 weight ratio of dye to polysaccharide more than 90% of the polysaccharide was found in the precipitate by means of a procedure described below for the isolation of the dye-binding polysaccharides. The dye did not precipitate arabic acid in 10% (w/v) NaCl or in 8 M-urea. Heating an aqueous solution of arabic acid in a boiling-water bath for 1 hr. destroyed its ability to be precipitated with the dye.

The following seed materials were used for isolation of the dye-binding polysaccharides: soya-bean flour was a defatted product marketed as Soyafluff 200 W by Central Soya, Chicago, Ill., U.S.A.; jack-bean flour was a defatted product marketed as jack-bean meal by Sigma Chemical Co., St Louis, Mo., U.S.A.; maize flour was obtained from seeds of variety Laguna F1, supplied by Hazera Seed Growing, Haifa, Israel. Extracts containing the dye-binding polysaccharides were prepared by stirring seed flour (100 g.) with cold 1% (w/v) NaCl solution (900 ml.) for 3 min. in a homogenizer. The extracts were then clarified in a refrigerated high-speed centrifuge. (Extracts of both maize and jack bean were heated in a boiling-water bath for 5 min., chilled and clarified again in the centrifuge.)

Precipitation of the polysaccharide from 500 ml. of clarified extract was effected by the addition of a solution of the glucoside dye to a final concentration of 0·2 mg./ml. The mixture was left for several hours at room temperature and the red precipitate that formed was collected by centrifugation, dissolved and reprecipitated twice to ensure removal of extraneous material. The dissolution was effected in 0·1 N-NaOH (400 ml.) and reprecipitation was achieved by neutralizing the alkaline solution with NaH_2PO_4. The final precipitate was dissolved in 50–80 ml. of an alkaline salt solution (10% NaCl in 0·1 N-NaOH) and was resolved into polysaccharide and dye on a column of Sephadex G-50 (Pharmacia, Uppsala, Sweden) equilibrated with the same solution. The polysaccharide emerged after the void volume of the column. The polysaccharide solution was freed of alkali and salt by gel filtration on a Sephadex G-50 column equilibrated with water. It was stored as a freeze-dried powder. Soya bean yielded 100 mg., jack bean 40 mg. and maize 20 mg. of the dye-binding polysaccharide fraction/500 ml. of extract.

The isolated polysaccharides were fully precipitable by the dye. Their carbohydrate content in percentage of dry weight as determined by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956) with galactose as standard was: soya bean, 80; jack bean, 70; maize, 82. In chromatography of acid hydrolysates of the isolated polysaccharides on Whatman no. 1 paper in butan-1-ol–acetic acid–water (25 : 6 : 25, by vol.) two major spots were revealed with alkaline $AgNO_3$ (Trevelyan, Procter & Harrison, 1950). Their R_F values corresponded to those of galactose and arabinose. The hydrolysate of soya-bean polysaccharide was also subjected to chromatography in ethyl acetate–pyridine–water (8 : 2 : 1, by vol.) and again the two major spots that were revealed as above migrated at the same rates as galactose and arabinose markers.

The polysaccharide from soya bean was further characterized. It was found to be polydisperse. A 1% (w/v) solution in 0·2M-NaCl had a major component with a sedimentation coefficient of 5·2s and a minor component with a sedimentation coefficient of 12·0s. It had one titrable acid group, with pK 3·2, per equivalent weight of 1500. Like arabic acid, its property of being precipitated with the dye could be prevented by 10% NaCl or 8M-urea and destroyed by heating at pH 3.

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