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Comments on the use of Blue Dextran in gel chromatography

Blue Dextran is a high-molecular-weight dextran fraction carrying a covalently linked chromophore. This polymer (Blue Dextran 2000) is reported by the manufacturers (Pharmacia Fine Chemicals, Inc.) to be excluded from all grades of Sephadex and has been recommended for checking the packing of gel columns[1] and for the determination of void volumes during calibration[2]. A recent report by WHITE AND JENCKS[3] that Blue Dextran binds certain proteins prompts the author to report some of his experiences with this material.

In a recent experiment a column (100 × 5 cm) was packed with Sephadex G-200 which had been swelled according to the method recommended by the manufacturers[4] in 50 m*M* Tris–HCl buffer pH 7.5–0.1 *M* NaCl. After pumping the column in an ascending direction for two days with the same buffer, a sample (10 ml) of Blue Dextran solution (10 mg/ml), which had been stored at room temperature for about six months was applied to the bottom of the column. An unusual phenomenon was observed, whereby the Blue Dextran failed to pass through the column. Despite pumping the column with several bed-volumes of buffer, all the Blue Dextran remained apparently bound to the gel within one in. of the bottom flow adapter. Communication with the suppliers yielded the information that this phenomenon had been reported before and generally took place when "aged" solutions of the chromogenic material were used.

After being assured that this would not interfere with chromatography on the column[5], a sample of a partially purified enzyme preparation (sweet-corn R-enzyme[6]) was applied to the column and elution continued. 185 fractions were collected, by which time we knew from previous experiments the activity should have been eluted, and the fractions assayed for enzymic activity. None was found, despite the fact that protein had been eluted (Fig. 1). On the basis that the only possible explanation for

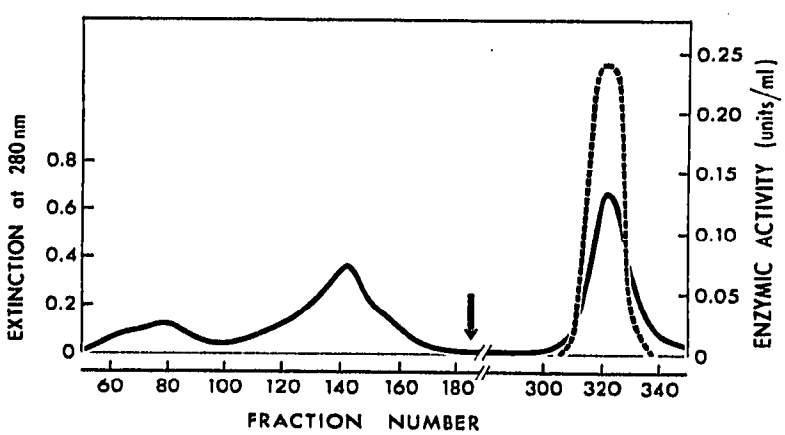

Fig. 1. Elution profile of a sweet-corn R-enzyme preparation chromatographed on Sephadex G-200 in the presence of Blue Dextran. The continuous line shows the distribution of protein in the fractions as measured by the extinction at 280 nm. The broken line shows the distribution of enzymic activity determined using pullulan as substrate[6]. Elution was carried out initially with 50 m*M* Tris–HCl buffer 7.5–0.1 *M* NaCl; at the point indicated by the arrow the NaCl concentration was increased to 0.5 *M*. The fractions were of volume 12 ml.

this was the presence of the Blue Dextran on the column, possibly acting as an ion-exchanger and binding the enzyme, the sodium chloride concentration in the eluting buffer was then increased to 0.5 *M*. A Uvicord connected in the outflow from the column showed a peak of protein which started to appear after another 120 fractions had been collected (Fig. 1). Assay for enzymic activity showed that the enzyme was in this protein peak; the recovery of activity was 100%. The Blue Dextran, however, remained bound to the Sephadex and the contaminated gel was then discarded.

It is difficult to explain the mechanism of adsorption of the Blue Dextran to the Sephadex. It is, however, clear that the Blue Dextran was able to bind proteins applied to the column and that the binding was inhibited at increased salt concentration. This suggests an ion-exchange type of mechanism, possibly through the sulfonic acid groups in the dye.

The important practical point which emerges from this is that extreme caution must be exercised when gel columns, on which Blue Dextran has been run, are used for chromatography of proteins. Accumulation of Blue Dextran is frequently noticed at the bottom (or top) of gel beds and also in the fine nylon mesh of sample applicators and flow adapters (despite filtration of the Blue Dextran solutions). The possibility of artefactual results is obvious from the experience described.

This problem has been overcome by discontinuing the use of Blue Dextran. Void volumes of columns are now determined in this Laboratory by use of proteins of sufficiently high molecular weight to be excluded from the gels. Column packings are checked using a solution of myoglobin. This latter protein is also useful for checking the packing of Biogel columns on which adsorption effects have been noticed when Blue Dextran is used, especially when the eluting buffer is of low ionic strength.

A possible application of this phenomenon is to enzyme purification, and we are investigating this possibility. Such an application of Blue Dextran, in a slightly different manner, has been suggested by WHITE AND JENCKS[3].

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