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Note

Isolation of synthetic enzyme–dextran conjugates by chromatography on concanavalin A–Sepharose

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Concanavalin A (Con A), the carbohydrate-binding protein from the jack bean, interacts with monosaccharides having α-D-mannosyl and α-D-glucosyl configurations, and oligosaccharides and polysaccharides containing non-reducing terminal monosaccharides with the same configuration[1]. Advantage has been taken of this property to isolate the lectin by chromatography on gels of cross-linked dextran (Sephadex), with which it interacts strongly, and from which it may be recovered by elution with an appropriate monosaccharide or glycoside, most commonly methyl-α-D-glucoside[2]. Con A has also been used in the immobilized form for isolation and separation of polysaccharides[3,4], and for the purification of naturally-occurring glycoproteins[5] and the glycopeptides produced therefrom by proteolytic degradation[6].

During studies on the synthesis and properties of enzyme–carbohydrate conjugates, prepared by covalent attachment of enzymes to soluble cyanogen bromide-activated dextran, the need arose for a simple and rapid procedure to assess the extent of coupling of enzymes with activated dextran, and to remove residual uncoupled enzyme from conjugate preparations. In this communication we describe the application of chromatography on Con A–Sepharose for this purpose.

EXPERIMENTAL

Dextran–enzyme conjugates were prepared by the procedures reported previously[7–11]. Con A–Sepharose (Pharmacia, Piscataway, N.J., U.S.A.) was packed in columns (8.0 × 0.5 cm I.D.) and washed with about 20 bed volumes of an appropriate buffer (see below). Samples of enzyme–dextran conjugate preparations (routinely 0.2 ml containing 0.2 mg enzyme and 2 mg dextran) were then applied to the column and eluted with the column buffer at 22°, fractions of volume 1.0 ml being collected automatically. After unbound material had emerged from the column, the eluting buffer was made 0.25 *M* with respect to methyl α-D-glucoside and elution continued, to recover bound material. Protein was located in the column fractions by measure-

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ment of ultraviolet absorbance at 280 nm; the fractions were also assayed for enzymic activity, and analyzed for carbohydrate by the phenol–sulfuric acid method[12] where appropriate. Fig. 1 shows the results of chromatography of a catalase–dextran conjugate preparation in this way. Chromatography of a mixture of catalase and dextran in the same manner resulted in elution of all enzyme activity in the fractions prior to the methyl-α-D-glucoside wash. The results of molecular-sieve chromatography of the same catalase–dextran conjugate and a simple mixture of its constituents have been shown elsewhere[11]. Dextran conjugates of a number of other enzymes, for example bacterial α-amylase[7] and trypsin[10] have also been chromatographed on Con A–Sepharose in a manner similar to that used for the catalase–dextran conjugate, and with the same results.

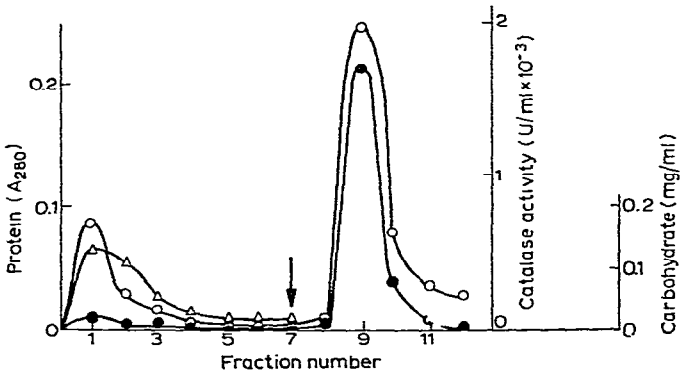

Fig. 1. Chromatography of catalase–dextran conjugate on a column (8.0 × 0.5 cm I.D.) of Con A–Sepharose. The sample applied contained 0.2 mg catalase and 2 mg dextran; elution was with 25 m*M* phosphate buffer, pH 7.0. At the point indicated by the arrow, methyl-α-D-glucoside was incorporated into the eluting buffer. ●, Catalase activity; ○, protein detected by absorbance at 280 nm; △, carbohydrate. Carbohydrate was not measured after fraction 7 because of the interference by methyl-α-D-glucoside.

DISCUSSION

When enzyme–dextran conjugates are chromatographed on columns on Con A–Sepharose, the majority of the enzyme activity binds to the immobilized lectin and may be recovered by washing with methyl-α-D-glucoside. Small amounts of activity, presumably representing the traces of unconjugated enzyme that nearly always remain in conjugate preparations, usually emerge from the column in the early fractions, prior to the methyl-α-D-glucoside wash. Hitherto these traces of unconjugated enzyme have been removed by gel filtration on columns of Sephadex or other molecular sieves[7–11]. The recovery of catalase activity after chromatography of catalase–dextran conjugates on Con A–Sepharose was generally 60–80% of the starting activity, this being only slightly lower than that recovered after molecular-sieve chromatography (90–100%). The recoveries of activity after chromatography of α-amylase and trypsin conjugates were also satisfactory.

Chromatography on Con A–Sepharose of the dextran used in the preparation of the conjugates showed that a substantial proportion of it failed to bind, presumably because of its substantially linear nature, branching being required for

strong interaction with Con A[13]. In spite of this we have found that most of the enzyme activity in enzyme–dextran conjugates is bound to the immobilized lectin, presumably because of the intramolecular cross-linking of dextran that takes place during the conjugation process[8,10].

Most experiments have been carried out using a buffer, 25 m*M* citrate-phosphate, pH 7.0, containing sodium chloride (150 m*M*) and the divalent cations Mn^{2+}, Mg^{2+} and Ca^{2+} (each 1 m*M*) required for the reactivity of Con A, as column eluent. The columns are washed with about 20 bed volumes of buffer between chromatography of successive samples and are re-usable many times. In other cases, for example when the divalent cations have an adverse effect on the activity or stability of the enzyme being chromatographed, as was found to be the case with catalase, chromatography may be satisfactorily performed using a buffer such as phosphate (25 m*M*, pH 7.0) for elution. In such cases, retention of maximum carbohydrate binding activity may be ensured by washing the adsorbent between runs with a buffer containing the divalent cations, followed by re-equilibration with buffer free from these ions.

The method described is extremely simple to perform and gives purified enzyme–dextran conjugates in 1–2 h, rather than the 15–25 h required when molecular-sieve chromatography is used. It is particularly appropriate when a large number of enzyme–dextran conjugate preparations are to be compared. It is also useful in cases where the enzyme being coupled has a high molecular weight, in which case clear-cut separation of free and conjugated enzyme is sometimes difficult. In all cases tested, analyses of the products prepared by the method described agree closely with the analyses after isolation by molecular-sieve chromatography. The procedure is likely only to be useful, however, in cases where the enzyme being conjugated does not itself contain carbohydrate since natural glycoproteins themselves bind to Con A–Sepharose[5].

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