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The use of concanavalin A in the purification or separation of multiple forms of brain hydrolases

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Abstract. Concanavalin A bound to Sepharose has been used for the purification of brain β-galactosidase, α-L-fucosidase, α-D-mannosidase, arylsulphatase and β-glucuronidase. Several factors *viz* pH, temperature and concentration of α-methyl glucoside influenced the binding and elution of these enzymes. A lysosomal acid α-mannosidase and a cytosolic neutral mannosidase were separable by concanavalin A-Sepharose chromatography. Similarly lysosomal and microsomal β-glucuronidases were separable using gradient elution with α-methyl glucoside. The results indicate the usefulness of this lectin for the isolation of wide variety of enzymes under specified experimental conditions.

Keywords. Concanavalin A; chromatography; brain hydrolases.

Introduction

Concanavalin A (Con A), a lectin derived from *Canavalia ensiformis* or *Canavalia gladiata* has found widespread use in the purification of several glycoproteins. It has a specificity towards binding α-D-mannopyranoside or α-D-ghicopyranoside structures containing unmodified C-3, C-4 and C-6 hydroxyl groups (Goldstein, 1976). An earlier observation made in this laboratory by Bishayee and Bachhawat (1974) indicated that several lysosomal enzymes of the brain tissue bind to Con A linked to Sepharose-4B. Following these observations, we found that Con A-Sepharose could be conveniently used for the purification or separation of multiple forms of brain lysosomal enzymes. In the present study we report our observations on the separation by affinity Chromatographie using Con A-Sepharose of β-D-galactosidase, α-L-fucosidase, α-D-mannosidase, arylsulphatase and β-D-glucuronidase of monkey brain.

Materials and methods

Con A was prepared from *C. gladiata* according to Surolia *et al.* (1973), and coupled to cyanogen bromide activated Sepharose 4-B as described by Cuatrecaas and Parikh (1972). The usual concentration of Con A bound to Sepharose was about 10 to 15 mg/ml gel. α-Methylglucoside and the *p*-nitrophenyl derivatives of the various glycosides, used as substrates, were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. Nitrocatechol sulphate was prepared according to Dodgson and Spencer (1957).

β-D-Galactosidase, α-L-fucosidase, α-D-mannosidase, arylsulphatase and β-D-glucuronidase were assayed as described earlier (Alam and Balasubramanian,

1978a, b; Lakshmi and Balasubramanian, 1980; Mathur and Balasubramanian, 1981; Alvares and Balasubramanian, 1982.

Protein was estimated according to Lowry *et al.* (1951).

Results

The experimental condition facilitating the binding to Con A-Sepharose and elution of the acid-glycosidases of monkey brain are summarised in table 1. The pH of binding, the pH of elution, the concentration of α-methylglucoside used for elution, and the temperature of elution affected the chromatography of these enzymes on the Con A-Sepharose.

Table 1. Experimental conditions for the binding and elution of brain enzymes to Con A-Sepharose.

Brain enzyme	Binding characteristics to Con A-Sepharose	Elution with α-methyl glucoside
β-D-Galactosidase	Binds at pH 6.0. Both galactosidases *A* and *B* bind	Elutable at 25°C. No significant elution at 4°C.
α-L-Fucosidase	Binds at pH 6.0. All the three forms of fucosidase bind	Elutable at 25°C
α-D-Mannosidase	The cytosolic neutral mannosidase does not bind but lysosomal acid mannosidase binds at pH 6.0	Elutable at 25°C
β-D-Glucuronidase	Both lysosomal and microsomal glucuronidases bind at pH 6.0	Both enzymes are eluted at 28°C. The lysosomal enzyme is eluted at low and the microsomal enzyme at high concentration of α-methyl glucoside

β-*Galactosidase*

β-D-Galactosidase was purified from monkey brain homogenate through five steps (Alam and Balasubramanian, 1978a), and a key step in the purification of the enzyme was Con A-Sepharose affinity chromatography. The enzyme eluted from this column upon subsequent Sepharose-6B gel filtration, resolved into a high molecular weight (β-galactosidase *B*), and a low molecular weight (β-galactosidase *A*) component. Galactosidase *B* was purified to apparent homogeniety as evidenced by sodium dodecyl sulphate (SDS) gel electrophoresis (Alam and Balasubramanian, 1978a). The *B* enzyme had a molecular weight of approximately 1.2×10^6 and the *A* enzyme about 1.2×10^5, ten times lesser than *B*. Both the enzymes were similar in their pH optima, K_m value for *p*-nitrophenyl β-galactoside and competitive inhibition by Y-D-galactonolactone. The specificity of the monkey brain β-galactosidases towards glycolipid substrates galactosylceramide, lactosylceramide and GMI ganglioside were somewhat similar to the findings of Tanaka and Suzuki (1977).

α-*L-Fucosidase*

An ammonium sulphate fraction of this enzyme from monkey brain could be purified about 170 fold in a single step by Con A-Sepharose chromatography (Alam and Balasubramanian, 1978b). The enzyme was bound at pH 6.0 to the Con A-Sepharose and eluted with α-methyl glucoside at 25°C. The purified enzyme on Sephadex G-200 gel filtration gave evidence for three peaks of enzyme activity. These three enzyme activities differed not only in their molecular weight, but also in their pH optima and thermal stability (Alam and Balasubramanian, 1978b).

α-*D-Mannosidase*

This enzyme was shown to be present in the monkey brain in two forms, an acid mannosidase present in the lysosomal fraction and a neutral mannosidase present in the cytosol. These two enzymes could be conveniently separated from each other by Con A-Sepharose chromatography (Mathur and Balasubramanian, 1981). While the acid mannosidase totally bound to the column (and eluted by α-methyl glucoside) the neutral enzyme did not bind to the column. It is presumable that the difference in carbohydrate composition of the two forms of enzymes might be responsible for their behaviour. The acid and neutral mannosidases were found to differ also in their response to metal ion activation, pH optima, thermal stability and molecular weight (Mathur and Balasubramanian, 1981).

Arylsulphatases

The arylsulphatase *A, B* and B_m of brain could be separated from one another by DEAE-cellulose column chromatography (Lakshmi and Balasubramanian, 1980). All these enzymes bound to Con A-Sepharose could be eluted with α-methyl glucoside. An unusual property exhibited by the arylsulphatase B_m was, that after Con A-Sepharose chromatography, it was totally and irreversibly bound by Sephadex G-200 (Lakshmi and Balasubramanian, 1980).

β-*D-Glucuronidases*

The monkey brain contained a lysosomal and a microsomal glucuronidase as observed in other tissues (Tulsiani *et al.,* 1978). They had similar pH optima and both of them bound to Con A-Sepharose. They could be partially separated from each other by using gradient elution with α-methyl glucoside. It is possible that the two glucuronidases differ in their glucose or mannose content resulting in their differential elution pattern (Alvares and Balasubramanian, 1982).

Discussion

The above results indicate that Con A bound to Sepharose can be most conveniently used not only for the isolation and purification of the lysosomal acid glycosidases of the brain, but also for the separation of the non-lysosomal and neutral glycosidases from the acid glycosidases. Use of this lectin-Sepharose has also been found in getting a concentrated form of the lysosomal enzymes from body fluids such as cerebrospinal fluid (Cherian and Balasubramanian, 1978). In summary Con A-Sepharose can be used; (a) for the purification of the lysosomal enzymes,

(b) for getting a concentrated form of these enzymes from body fluids, and(c) for a preliminary assessment of the carbohydrate composition of these enzymes.

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