THE INTERACTION OF DOPAMINE-β-HYDROXYLASE WITH CONCANAVALIN A AND ITS USE IN ENZYME PURIFICATION

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SUMMARY

Dopamine-β-hydroxylase forms a complex with concanavalin A and can be quantitatively dissociated from the complex with α-methyl-D mannoside. It can thus be separated from other chromaffin vesicle proteins that have no affinity for the lectin. Using this observation it was possible to purify the enzyme by a single passage through a column of concanavalin A-Sepharose. Analysis of the concentrated eluate by disc gel electrophoresis showed that the dopamine-β-hydroxylast was 93% pure. The binding of this glycoprotein enzyme to concanavalin A indicates that the polysaccharide moiety is highly branched and contains α-D-mannopyranosyl and/or α-D-glucopyranosyl residues as the terminal sugars.

The enzyme dopamine-β-hydroxylase (EC. 1.14.17.1) is found in subcellular organelles in both the adrenal medulla and sympathetic nerves and is released from these cells upon neural stimulation. A recent report by Wallace et al. (1) has demonstrated the presence of a carbohydrate moiety associated with dopamine-β-hydroxylase isolated from bovine adrenal glands. Since mannose and glucose accounted for a large proportion of the total sugar content, it was of interest to examine whether the enzyme could be isolated by affinity chromatography using concavanalin A. This plant lectin has been shown to specifically bind α-D-mannosyl, α-D-glucosyl and sterically similar residues that are located on the free terminal of a carbohydrate chain (2). This communication describes a rapid and simple procedure for the purification of dopamine-β-hydroxylase on an affinity column of concanavalin A bound to sepharose.

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METHODS

Dopamine-β-hydroxylase activity was assayed by the method of Nagatsu and Udenfriend (3). Tyramine is used as a substrate and the results are reported in units/ml. One unit is equivalent to one μmole of octopamine formed per minute. Protein estimations were performed using the procedure of Lowry et al. (4). Analysis of the purified enzyme was carried out on polyacrylamide gels according to the method described by Fishbein (5). Concanavalin A-Sepharose was purchased from Pharmacia (Sweden).

The purification of the enzyme was carried out at 2^{o}. Highly purified chromaffin vesicles were prepared by the method of Smith and Winkler (6) from four fresh bovine adrenal glands.

RESULTS

The sedimented chromaffin vesicles were lysed in 10 volumes of 5 mM potassium phosphate buffer, pH 6.5, using a loosely fitting teflon-glass homogenizer. The vesicle membranes were removed by centrifugation at 40,000 x g for 30 min. The supernatant solution was diluted with an equal volume of 100 mM phosphate buffer, pH 6.5, containing 0.4 M sodium chloride. This lysate was then applied to a column of concanavalin A-Sepharose (4 x 0.7 cm) previously equilibrated with 50 mM phosphate buffer, pH 6.5, containing 0.2 M sodium chloride. The flow rate was kept at approximately 0.3 ml/min.

After the enzyme had been absorbed to the column, it was washed extensively with buffer until the absorbance of the effluent at 280 nm was less than 0.005. Dopamine-β-hydroxylase was eluted with the same buffer containing 10% α-methyl-D-mannoside. As the elution appeared to be time dependent, the flow rate was adjusted to approximately 0.1 ml/min so that the enzyme could be collected in a maximum of twenty column volumes. The pooled eluate containing dopamine-β-hydroxylase was concentrated by ultrafiltration in an Amicon Diaflo apparatus using an XM100A membrane. After

dialysis against 50 mM phosphate buffer, pH 6.5, containing 0.1% sodium azide, the purified enzyme was stored at 2°.

The specific activity of the dopamine-β-hydroxylase present in the lysate of the chromaffin vesicles was 0.08 units/mg protein. The total amount of activity applied to the column was 8.5 units of which 99% was bound. The recovery of enzymatic activity from the column was 88%, with a 67-fold purification. The eluted enzyme had a specific activity of 5.4 units/mg protein (see Table 1) that was not altered by the concen-

TABLE 1. PURIFICATION OF DOPAMINE-β-HYDROXYLASE USING CONCANAVALIN A

Fraction	Total Enzyme Activity (units)	Total Protein (mg)	Specific Activity (units/mg)
Chromaffin vesicle lysate	8.5	102.8	0.08
Eluate from concanavalin-A column	7.5	1.4	5.4

The lysate from chromaffin vesicles isolated from four bovine adrenal glands was applied to a column of concanavalin-A Sepharose (4 x 0.7 cm). After extensive washing the dopamine-β-hydroxylase was eluted with a 10% solution of α-methyl-D-mannoside in 50 mM potassium phosphate buffer.

tration procedure. Comparable specific activities have been reported previously for preparations from the same source obtained by purification procedures requiring a number of steps (see for example refs. (7-9)). When 10 μg of the purified enzyme was analyzed by disc gel electrophoresis only a single band could be seen. However with 40 μg of the preparation, two minor bands could be distinguished. Spectrophotometric analysis of

the stained gels (5) showed that the two minor bands accounted for 4% and 3% of the total protein with 93% in the dopamine-β-hydroxylase band (see Figure 1).

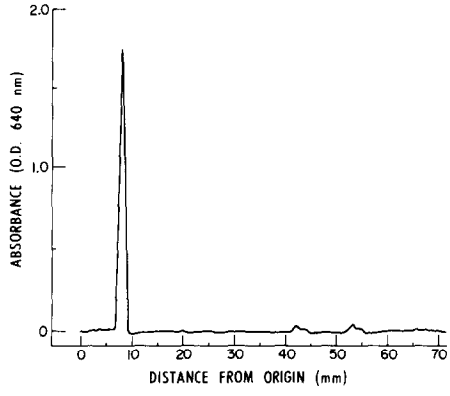

Figure 1. Analysis of concanavalin A purified dopamine-β-hydroxylase by disc gel electrophoresis. Forty μg of protein from the concentrated eluate from a column of concanavalin A-Sepharose was applied to a 7.5% acrylamide gel and subjected to electrophoresis. The absorbance of the stained gel is shown.

DISCUSSION

The use of concanvalin A for the isolation of dopamine-β-hydroxylase from fresh bovine adrenal glands allows a rapid purification of the enzyme with almost no loss of catalytic activity, in contrast to more classical procedures. In addition the high capacity of the concanavalin A-Sepharose enables the isolation of purified dopamine-β-hydroxylase on a much larger scale. Its greatest value, however, may prove to be its applicability to the isolation of the enzyme from sources that do not permit the separation of the chromaffin vesicles, such as from frozen tissue or from serum. In a preliminary experiment with a homogenate of human adrenal glands obtained at autopsy, we achieved a 70-fold purification of the enzyme after a single passage through a column of the concanavalin A-Sepharose.

The method should also allow the preparation of antigenically pure dopamine-β-hydroxylase for the production of specific antiserum to the

enzyme. This could best be achieved by following the concanavalin A purification with disc gel electrophoresis in the manner described by Hartman and Udenfriend (10).

Affinity columns with lectins, such as concanavalin A, may also be useful for removing glycoproteins from preparations of other soluble proteins of the chromaffin vesicles, and in particular for chromogranin A. This vesicular protein, when purified, generally contains a small amount of dopamine-β-hydroxylase as a contaminant (11). Although the dopamine-β-hydroxylase present usually accounts for only a small percentage of the total protein e.g. <1%, it has proved troublesome to remove, and the highly antigenic nature of the enzyme interferes with the production of a mono-specific antiserum against the chromogranin A. Our observation that chromogranin A does not bind to concanavalin A should allow the complete removal of the contaminating dopamine-β-hydroxylase from such preparations.

The present finding supports the recent claim that dopamine-β-hydroxylase is a glycoprotein (1). The high affinity of dopamine-β-hydroxylase for concanavalin A would also predict that the polysaccharide moiety is highly ramified, and that many of the chains contain α-D-manno-pyranosyl and/or α-D-glucopyranosyl residues as the terminal sugars (12).

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