CONCANAVALIN A BINDS TO PURIFIED PROLYL HYDROXYLASE AND PARTIALLY INHIBITS ITS ENZYMIC ACTIVITY

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SUMMARY

Prolyl hydroxylase [(EC 1.14.11.2; prolyl-glycyl peptide, 2-oxoglutarate dioxygenase (4-hydroxylating)] was electrophoresed on polyacrylamide gels and the enzyme in the gels was shown to bind [acetyl-^{3}H]concanavalin A. The enzyme-lectin complex was dissociated by treating the gel with methyl α-D-mannopyranoside, a sugar known to inhibit binding of concanavalin A to glycoproteins. Furthermore, prolyl hydroxylase activity was partially inhibited by concanavalin A when the enzyme was assayed in the absence of bovine serum albumin, a protein which enhances enzymic activity. The inhibition of enzyme activity was prevented by sugars known to react with concanavalin A.

INTRODUCTION

Prolyl hydroxylase catalyzes the synthesis of trans-4-hydroxyproline in collagen by the hydroxylation of prolyl residues in unassembled proα chains of procollagen (1-4). The enzyme has been purified from a variety of sources and has been shown to be a tetramer consisting of two different types of subunits with molecular weights of about 60,000 and 64,000 (5,6).

In order to obtain further structural information on prolyl hydroxylase, experiments were undertaken here to determine whether or not prolyl hydroxylase is a glycoprotein. Concanavalin A binds

to sugars such as to α-D-mannopyranoside, α-D-glucopyranoside and D-fructofuranoside (7,8), and the binding properties of this lectin have been used as a tool to isolate and to characterize sugar-containing biopolymers, such as polysaccharides, glycolipids and glycoproteins (8-12). In the present study, we have used concanavalin A to demonstrate that purified prolyl hydroxylase contains carbohydrate.

MATERIALS AND METHODS

Jack bean concanavalin A (3 x crystallized under ammonium sulfate) was purchased from Miles Laboratories, Kankakee, Illinois. The lectin was dissolved in 1 M NaCl, centrifuged at 20,000 x g for 30 min and the supernatant was dialyzed against 0.1 M NaCl at 4°C before use. The extinction coefficient for concanavalin A with a 1-cm light path, $E^{1\ mg/ml}_{280\ nm}$, was 1.30 (13). D-Glucose and D-fructose were purchased from Fisher Scientific Co., Fair Lawn, New Jersey. D-Galactose, D-mannose, methyl α-D-glucopyranoside, catalase and Coomasie brilliant blue R were obtained from Sigma Chemical Co., St. Louis, Missouri. Dithiothreitol was purchased from Calbiochem, San Diego, California, and poly(L-Pro-Gly-L-Pro) with a molecular weight of about 2,400 was obtained from Miles-Yeda, Ltd., Rehovot, Israel. [Acetyl-^{3}H]concanavalin A, α-keto[1-^{14}C]glutarate and Protosol were purchased from New England Nuclear, Boston, Massachusetts. Custom Cocktail (62.5% LSC complete, 37.5% methyl cellosolve) was obtained from Yorktown Research, South Hackensack, New Jersey.

Prolyl hydroxylase was purified from chick embryo homogenates by affinity column chromatography as previously reported (5,14). The enzymic reaction was carried out in the presence of α-keto[1-^{14}C]glutarate and the amount of [^{14}C]CO_2 evolved in the reaction was measured as described elsewhere (5,15). The assay was carried out in a final volume of 1.0 ml which contained 3 to 5 μg of purified enzyme, 250 μg of Poly(L-Pro-Gly-L-Pro), 0.05 mM $FeSO_4$, 2 mM ascorbate, 0.1 mM dithiothreitol, 0.2 mg of catalase, 0.1 mM α-keto[1-^{14}C] glutarate (adjusted to a final specific activity of 340 dpm per nmole), and 50 mM Tris-HCl buffer adjusted to pH 7.8 at 25°C. In some of the experiments 2 mg/ml of bovine serum albumin was included in the reaction mixture. The samples were incubated at 37°C for 20 min and the enzymic reaction was stopped with 1 ml of 1 M potassium phosphate, pH 5.0.

Disc electrophoresis of the native enzyme was carried out on 7% polyacrylamide cylindrical gels containing 89 mM Tris (hydroxymethyl)aminomethane, 89 mM boric acid and 2.5 mM disodium ethylenediaminetetraacetate, pH 8.28 (16). Thirty μg of purified enzyme per gel was applied directly to the polyacrylamide gel and then subjected to electrophoresis at 2 mA per gel for 4 hr at 6°C in a Buchler Polyanalyst temperature-regulated apparatus. One gel was stained with Coomasie brilliant blue and then scanned at 500 nm in a Gilford Model 240 spectrophotometer. Two other gels were incubated at 4°C

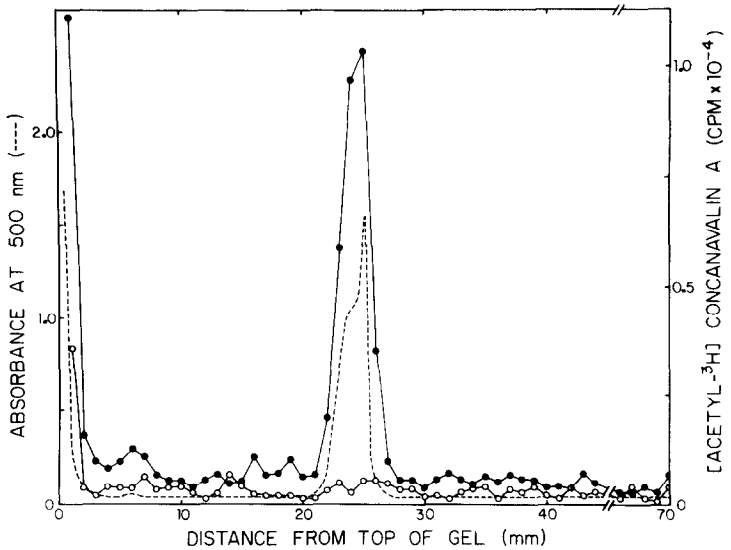

Fig. 1 Polyacrylamide gel electrophoresis of purified prolyl hydroxylase. Conditions for electrophoresis were as indicated in Materials and Methods. One gel was stained for protein (---). A second gel was incubated with [acetyl-^{3}H]concanavalin A and then washed with enzyme buffer (●——●). A third gel was incubated with [acetyl-^{3}H]concanavalin A and then washed with enzyme buffer containing 0.2 M of methyl α-D-mannopyranoside (o——o).

for 48 hr with 5 μCi of [acetyl-^{3}H]concanavalin A per ml of 0.2 M NaCl, 0.2 M glycine and 0.01 M Tris-HCl buffer, pH 7.8 (enzyme buffer) After incubation, excess radioactivity was removed from the gels by washing for 48 hr at 4°C. One gel was washed with 0.2 M NaCl, 0.2 M glycine and 0.01 M Tris-HCl buffer, pH 7.8, and the other gel was washed with the same enzyme buffer containing 0.2 M methyl α-D-mannopyranoside. The gels were then sliced into 1 mm slices with the Mickler gel slicer (Brinkmann Instruments, Inc., Westbury, New York), placed in a vial, and allowed to swell overnight at 60°C in 0.4 ml of a solution containing Protosol:H_2O:toluene (9:1:10). The samples were cooled, 20 ml of scintillation counting solution (Custom Cocktail) was added to each vial, and the samples were counted in an Intertechnique Model SL30 liquid scintillation spectrometer.

RESULTS

Purified prolyl hydroxylase was recovered as a single band of protein when examined by polyacrylamide gel electrophoresis in Tris-borate buffer, pH 8.28 (Fig. 1). Previous observation (5) indicated

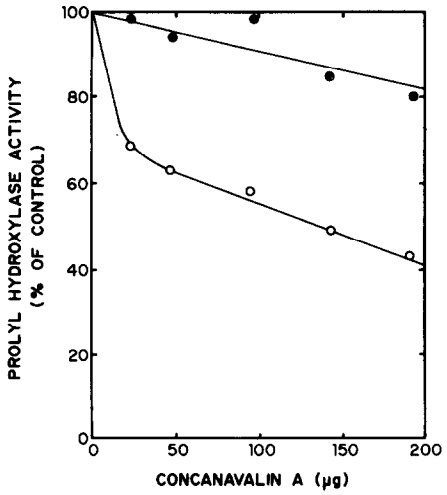

Fig. 2 Inhibition of prolyl hydroxylase activity by concanavalin A. Experimental conditions were as indicated in Materials and Methods. Enzymic activity was assayed in the presence of bovine serum albumin (●——●) or in the absence of bovine serum albumin (o——o).

that the protein in the band was enzymically active and consisted of the tetrameric form of the enzyme. The stained band was asymmetric (Fig. 1), apparently because relatively large amounts of protein were applied. When duplicate gels were incubated with [acetyl-^{3}H]concanavalin A, the lectin was bound to the region which contained the enzyme. The binding appeared to be specific, since the [acetyl-^{3}H]-concanavalin A was released from the gel (Fig. 1) by washing the gel with a solution containing methyl α-D-mannopyranoside, a sugar which binds to the lectin (7,8).

Further experiments demonstrated that when prolyl hydroxylase activity was assayed in the presence of bovine serum albumin, little inhibition was observed with up to 200 μg/ml of concanavalin A (Fig. 2). However, when bovine serum albumin was omitted from the incubation reaction, a marked inhibition was observed with the same concentrations of concanavalin A (Fig. 2). Enzymic activity was not affected by addition of any of several sugars to the reaction

TABLE I: Effect of concanavalin A and sugars on prolyl hydroxylase activity *

SUGAR	concentration (mM)	Minus Con A[1] (CPM)	Plus Con A (CPM)	Inhibition by Con A (%)
None	-	1659	793	52
D-Galactose	10	1854	691	63
	20	1652	913	45
D-Fructose	10	1660	1456	12
	20	1276	1360	0
Methyl α-D-glucopyranoside	10	1699	1553	9
	20	1758	1801	0
D-Mannose	10	1599	1511	6
	20	1652	1462	12
D-Glucose	10	1904	1923	0
	20	1966	2093	0
Methyl α-D-mannopyranoside	10	1679	1935	0
	20	1808	1990	0

* Prolyl hydroxylase activity was assayed as described in Materials and Methods. The concentration of concanavalin A was 356 µg/ml.

Abbreviation: [1]Con A, concanavalin A.

system (Table I). However, inhibition of prolyl hydroxylase activity by concanavalin A was prevented by several sugars which bind to the lectin (7). In contrast, no effect was obtained with galactose, a sugar which does not bind to concanavalin A (17).

DISCUSSION

The results presented here demonstrate that concanavalin A binds to and partially inhibits the enzymic activity of prolyl hydroxylase, and these observations strongly suggest that the enzyme contains carbohydrate. They do not exclude, however, the possibility that purified prolyl hydroxylase may have carbohydrate tightly associated but not covalently bound to the enzyme. There is no apparent explanation as to why bovine serum albumin, which enhances enzymic activity by an unknown mechanism (1,4), protects against inhibition by the lectin. The protection afforded by albumin may be explained by the presence of small amounts of carbohydrate in the commercial preparation of bovine serum albumin used here, or by some as yet unknown effect of the albumin on the enzyme.

Many intracellular proteins lack carbohydrate whereas many extracellular proteins contain carbohydrate, and it has been suggested (18-20) that carbohydrate may play a specific role in the processing of proteins synthesized for export from cells. Prolyl hydroxylase has been located in the cisternae of the rough endoplasmic reticulum of cells synthesizing procollagen (21,22), and apparently is not secreted from the cells (22). The presence of carbohydrate in prolyl hydroxylase may be important for either its location or its function in the rough endoplasmic reticulum.

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