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AFFINITY ADSORPTION OF L-GLUTAMINE D-FRUCTOSE-6-PHOSPHATE AMINOTRANSFERASE TO SEPHAROSE COUPLED WITH p-CHLOROMERCURIBENZOATE

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SUMMARY

L-Glutamine D-fructose-6-phosphate aminotransferase (E.C. 2.6.1.16) from mouse sublingual gland and small intestine was found to be adsorbed to a column of Sepharose coupled with p-chloromercuribenzoate. The enzyme could be eluted with mercaptoethanol, cysteine, glutathione or dithiothreitol with good recovery of the activity. About 50-fold purification of the enzyme in an extract of the sublingual gland, with its complete separation from glucosephosphate isomerase was achieved by eluting the column with a linear gradient of 0 to 20 mM dithiothreitol. It is suggested that the enzyme is not modified during the purification.

The activities of glutamine fructose-6-phosphate aminotransferase in the liver (1), intestine (2) and mammary gland (2) are significantly affected by various physiological conditions, such as tumor bearing, glucose feeding and pregnancy. Since the aminotransferase is believed to function in the biosynthesis of aminosugars (3-5), its activity should be important in regulating mucopolysaccharide biosynthesis. This possibility is supported by the fact that uridinediphospho-N-acetylglucosamine (UDP-N-acetylglucosamine), a metabolite produced by the aminotransferase, inhibited the enzyme activity (6) in a complicated way (7,8). For further studies on the properties and physiological role of the aminotransferase, a highly purified enzyme preparation is required.

The enzyme in liver can be purified 240- to 370- fold by ammonium sulfate precipitation, and DEAE-Sephadex and hydroxyapatite chromatographies

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(7,8), but since the enzyme is unstable (1, 3-8), a quicker and simpler procedure is required for its purification. This paper describes a simple rapid purification procedure using Sepharose coupled with p-chloromercuribenzoate (p-CMB Sepharose) as an affinity adsorbent.

MATERIALS AND METHODS

p-CMB Sepharose was prepared essentially as described by Cuatrecasas (9). By this procedure about 6 μmoles of p-CMB per ml of gel was coupled, as estimated by measuring the uptake of cysteine with 5,5-dithiobis (2-nitrobenzoic acid) (9,10).

Glutamine fructose-6-phosphate aminotransferase activity was assayed by the method of Pogell (11), following the procedure described previously (12). Unless otherwise stated, the standard reaction mixture for activity assay was composed of 10 μmoles glutathione (reduced form), 1 μmole EDTA, 15 μmoles L-glutamine, 10 μmoles fructose-6-phosphate, and 40 μmoles sodium phosphate buffer (pH 7.5). Glucosephosphate isomerase (E.C. 5.3.1.9) activity was assayed by the method of Slein (13). One unit of enzyme is defined as the amount causing formation of 1 μmole of product per min.

The sublingual gland and small intestine were used as enzyme sources, because these organs have high enzyme activities (2,12). The sublingual glands and small intestine (ileum) from ICR strain mice were homogenized with 50 mM sodium phosphate buffer (pH 7.5) containing 0.1 M KCl and 1 mM EDTA (concentrations of homogenates: 2% for sublingual gland and 10% for small intestine), and centrifuged at 105,000 × g for 1 h, and the resulting supernatants were used for experiments.

RESULTS AND DISCUSSION

The extracts of the sublingual gland and small intestine were applied to 1 ml-columns of p-CMB Sepharose. The columns were washed with the equilibrating buffer (50 mM sodium phosphate buffer containing 0.1 M KCl and 1 mM EDTA), and eluted with the same buffer containing 50 mM concentrations of various SH-reagents. The absorbance at 280 nm and the enzyme activity of the washing fluid and eluate were measured. As shown in Table I, about half the protein in the extracts was not adsorbed, but most of the enzyme activity was adsorbed to the column. The enzyme activity could be recovered almost completely by elution with various SH-reagents, although some variation in recovery was observed with different SH-reagents.

These results suggest that the aminotransferase can be highly purified by p-CMB Sepharose column chromatography, since the adsorption of the enzyme

TABLE I RECOVERIES OF THE AMINOTRANSFERASE ACTIVITY AND PROTEIN FROM A p-CMB SEPHAROSE COLUMN WITH VARIOUS SH-REAGENTS

SH-Reagent	Sublingual gland		Small intestine	
	Protein	Activity	Protein	Activity
Dithiothreitol	55	114	54	108
Mercaptoethanol	64	89	52	88
Cysteine	69	76	55	79
Glutathione	45	86	43	93

Samples of 1 ml, containing 161 munits/ml (A_{280}nm=5.31) from sublingual gland or 80 munits/ml (A_{280}nm=9.18) from small intestine were applied to columns of p-CMB Sepharose (bed volume, 1 ml). Columns were washed with 3 ml of equilibration buffer, and adsorbed materials were eluted with 4 ml of solution of SH-reagent (50 mM) in equilibration buffer. Percentage recoveries in eluates from each column of applied samples are shown.

seems to be due to the affinity of SH residues in the enzyme molecule to p-CMB of the resin.

Since the highest recovery of enzyme activity was obtained by elution with dithiothreitol (Table I), we attempted to purify the enzyme in the extract of sublingual glands by elution with a linear gradient of dithiothreitol (Fig. 1). The activity of glucosephosphate isomerase, in each fraction of eluate was also measured, because its separation from the aminotransferase is important for studies on the properties of the aminotransferase (7) (see also below).

Fig. 1 shows the typical elution profiles of the aminotransferase, glucosephosphate isomerase and protein. Most of the protein adsorbed to the column was eluted with a low concentration of dithiothreitol but the aminotransferase was eluted with a high concentration. Glucosephosphate isomerase was recovered in the unadsorped fraction and in the eluate with a low dithiothreitol concentration (97 and 3%, respectively). Therefore, a purification of about 50-fold of the aminotransferase (fractions 27 and 28) was accomplished with complete removal of glucosephosphate isomerase.

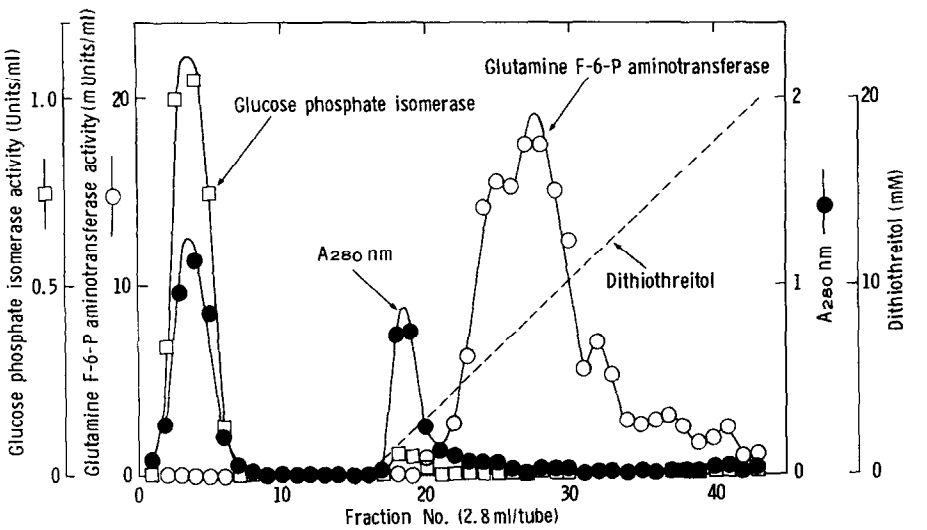

Fig. 1 Affinity chromatography of glutamine fructose-6-phosphate aminotransferase from the sublingual gland on a p-CMB Sepharose column. A sample (8.6 ml) containing 65.5 munits/ml of the aminotransferase and 1.3 units/ml of glucosephosphate isomerase was applied to a column of p-CMB Sepharose (bed volume, 3 ml). The column was washed with 30 ml of equilibration buffer and enzymes were eluted with a linear gradient of dithiothreitol (0-20 mM) in the same buffer. The recoveries of the aminotransferase, glucosephosphate isomerase and protein were 104%, 95% and 100% respectively. ○ , Aminotransferase; □ , glucosephosphate isomerase; ● , protein (A_{280}nm).

The effects of various inhibitors on the purified enzyme and the crude enzyme were compared (Table II). The purified enzyme was inhibited by UDP-glucose and p-CMB to the same extents as the crude enzyme. It was inhibited less than the latter by UDP-N-acetylglucosamine. This difference may be due to the presence of glucose-6-phosphate in the reaction mixture with crude enzyme, since this would be formed by contaminating glucosephosphate isomerase during incubation (7). This possibility was confirmed by the results that the purified enzyme was inhibited by UDP-N-acetylglucosamine in the presence of glucose-6-phosphate (Table II). These properties of the enzyme resemble those of the liver enzyme reported previously (7,8). Therefore, the present procedure does not seem to modify the enzyme, since the enzyme purified by p-CMB Sepharose retained the properties of the crude enzyme of being inhibited by p-CMB and UDP-glucose, and by UDP-N-acetylglucosamine if glucose-6-phosphate was present (Table II).

TABLE II INHIBITIONS BY UDP-N-ACETYLGLUCOSAMINE, UDP-GLUCOSE, AND p-CMB OF PURIFIED AMINOTRANSFERASE FROM THE SUBLINGUAL GLAND

Inhibitor (mM)	Activity (nmoles/min/ml)	
	Crude	Purified
Exp. 1		
None	97.9 (100)	19.1 (100)
UDP-N-Acetylglucosamine (0.025)	51.9 (53)	16.6 (87)
UDP-N-Acetylglucosamine (0.10)	12.7 (13)	12.4 (65)
Glucose-6-phosphate (10)	—	18.4 (96)
UDP-N-Acetylglucosamine (0.10) + glucose-6-phosphate (10)	—	4.6 (24)
UDP-Glucose (5)	61.7 (63)	12.2 (64)
Exp. 2*		
None	51.6 (100)	8.2 (100)
p-CMB (0.02)	—	0.52(6.4)
p-CMB (0.05)	3.1 (6)	0.22(2.7)

A crude extract from the sublingual gland and enzyme purified on p-CMB Sepharose (fractions No. 25-29 in Fig. 1) were used. Values are means of two determinations. Values in parentheses are percentages of the control values.
* For estimation of inhibition by p-CMB, samples were passed through columns of Sephadex G-25 equilibrated with 50 mM sodium phosphate buffer containing 0.1 M KCl and 1 mM EDTA. Glutathione was omitted from the reaction mixture for this experiment.

The present results show that glutamine fructose-6-phosphate aminotransferase of the sublingual gland and small intestine can be purified highly by p-CMB Sepharose column chromatography with good recovery. Since the procedure is based on the affinity of the enzyme for p-CMB and since aminotransferases of mammalian origin are in general inhibited by low concentrations of p-CMB (14) (Table II), this procedure will be useful in purification of the aminotransferase from various tissues.

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