PURIFICATION OF β-GALACTOSIDASE FROM WHEAT-GERM BY AFFINITY CHROMATOGRAPHY

Hideaki HAMAZAKI and Kyoko HOTTA

Department of Biochemistry, Kitasato University School of Medicine, Sagamihara, Kanagawa 228, Japan

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1. Introduction

Affinity chromatography has been proved to be an excellent method to purify enzymes and other biomolecules [1]. For isolation of *Escherichia coli* β-galactosidase Steers et al. developed an adsorbent consisting of agarose gel coupled with β-thiogalactoside as a ligand via spacer arms [2]. In the case of this adsorbant, however, the adsorption phenomenon was pointed out not to be ligand-specific and appropriate spacer arms devoid of the ligand could bind *E. coli* β-galactosidase [3]. Another type of ligand, *p*-aminophenyl β-D-glycoside, was used for isolation of glycosidases from bovine and murine liver, but resolution of the enzymes was difficult because these enzymes possessed similar affinity to the binding glycones [4]. Therefore, it would be necessary to investigate a new ligand to develop a specific adsorbent for β-galactosidase.

In this paper we describe a new affinity method utilizing lactose-coupled polyacrylamide gel for purification of β-galactosidase from wheat-germ. The desired enzyme was adsorbed specifically to this adsorbent and eluted with the ligand.

2. Materials and methods

Wheat-germ (type 1) was purchased from Sigma, Bio-Gel P-300 (50–100 mesh) was from Bio-Rad Laboratories, and Sephadex G-150 (fine) was from Pharmacia. All chemicals were reagent grade. Galactosyl β-(1–4)glucitol was prepared by reduction of lactose with sodium borohydride.

2.1. *Enzyme assay*

Enzyme activity was assayed at 30°C in a mixture containing 50 μl of enzyme and 1 ml 0.4 mM *p*-nitrophenyl glycoside (Sigma) in 0.05 M sodium citrate buffer (pH 4.7). The reaction was terminated by the addition of 1.5 ml of 0.2 M sodium carbonate, and the amount of released *p*-nitrophenol was estimated spectrophotometrically using its molar extinction coefficient at 400 nm, $1.83 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$. One unit β-galactosidase was defined as the amount of enzyme which hydrolyzed 1 μmol substrate/min under the condition described above. Protein was determined by the method of Lowry et al. [5].

2.2. *Coupling of lactose to polyacrylamide gel*

Hydrazide derivative of Bio-Gel P-300, prepared by the method of Inman et al. [6], was suspended in 0.1 M lactose. The gel was heated in a boiling water bath for 1 h and stood overnight at room temperature. Free ligand was removed subsequently by washing the gel exhaustively with the column buffer consisting of 0.02 M sodium acetate buffer (pH 3.9) containing 0.1 M NaCl. Ligand 27–30 μmol was usually coupled to 1 ml packed gel.

2.3. *Enzyme preparation*

Enzyme preparation was performed at 4°C. A 200 g portion of wheat-germ was homogenized in 2 l of 0.1 M NaCl and adjusted to pH 3.9 with 1 N HCl. After centrifugation at 10 000 × *g* for 1 h, solid ammonium sulfate was added to the supernatant obtaining 60% saturation. The precipitate was collected by centrifugation, dissolved in 300 ml column buffer and dialyzed against the same buffer.

The crude enzyme fraction was applied on an affinity column (2.5 X 16 cm). The column was eluted with 300 ml/column buffer and was subsequently eluted with 200 ml same buffer containing 0.1 M galactosyl β (1—4) glucitol. The protein concentration of column effluent was estimated spectrophotometrically at 280 nm. The β-galactosidase fraction eluted with the ligand was concentrated to 5 ml using a Toyo UP-20 ultrafilter (Toyo Roshi Co.).

The β-galactosidase fraction was applied to a Sephadex G-150 column (2.6 X 95 cm) previously equilibriated with 0.05 M sodium phosphate buffer (pH 6.2) containing 0.2 M NaCl. Human γ-globulin, bovine serum albumin, ovalbumin, chymotrypsinogen A, and sperm-whale myoglobin were used as standards for molecular weight estimation of the purified enzyme.

2.4. *Gel electrophoresis*

The purity of the enzyme was examined by electrophoresis in 7.5% acrylamide gels using the procedure of Davis [7]. The gels were stained with Coomassie Blue R-250. In order to localize the enzyme activity parallel gels were sliced into 1 mm thickness and extracted with 0.05 M sodium citrate buffer (pH 4.7).

3. Results

3.1. *Ammonium sulfate fractionation*

Wheat-germ extracts contained several exoglycosidases, including α-mannosidase, β-galactosidase, α-galactosidase, β-*N*-acetylglucosaminidase, β-glucosidase and α-L-fucosidase. By ammonium sulfate fractionation the specific activity of β-galactosidase was increased 2-fold as shown in table 1, but all other glycosidases also remained in this fraction.

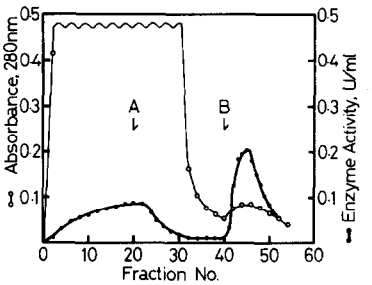

Fig.1. Purification of wheat germ β-galactosidase on polyacrylamide derivatized with lactose. Ammonium sulfate, 270 ml fraction, was applied on the column (2.5 X 16 cm), and after eluting with 300 ml column buffer (A). The column was eluted with 0.1 M galactosyl β-(1—4)glucitol in the same buffer (B). The flow-rate was 50 ml/h and 15 ml fractions were collected.

3.2. *Affinity chromatography*

The ammonium sulfate fraction was applied to the affinity column, and the glycosidases except β-galactosidase were eluted almost completely with the column buffer. The β-galactosidase adsorbed to the column was effectively eluted with 0.1 M galactosyl β-(1—4)glucitol (fig.1). The specific activity of β-galactosidase was elevated to 300-fold in this step. The cross-contamination of other glycosidases was removed almost completely. The column could be used repeatedly after washing with 0.1 M lactose and the column buffer.

3.3. *Gel-filtration and electrophoresis*

The enzyme fraction obtained by affinity chromatography was applied to a Sephadex G-150 column to remove protein contaminants devoid of enzyme activity (fig.2). In this step the specific activity increased again 5-fold. The molecular weight of the

Table 1
Purification of β-galactosidase of wheat-germ

Purification step	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	Recovery (%)
1. Extracts	12800	109.8	0.0086	1	100
2. Ammonium sulfate fraction	3240	68.0	0.021	2.4	62
3. Affinity chromatography	4.55	27.3	6.0	698	25
4. Gel-filtration	0.83	25.5	30.6	3560	23

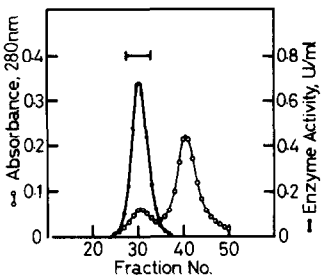

Fig.2. Gel-filtration of wheat-germ β-galactosidase. Enzyme fraction, 5 ml, obtained by affinity chromatography, was applied on the Sephadex G-150 column (2.6 × 95 cm). The column was eluted at a flow-rate of 13 ml/h and 10 ml fractions were collected. Tubes 28–33 were pooled as the purified β-galactosidase.

enzyme was estimated to be 7.5×10^4 by the gel-filtration analysis. Polyacrylamide gel electrophoresis of the purified enzyme indicated two bands which appeared to be associated with β-galactosidase activity (fig.3).

4. Discussion

Affinity chromatography is a very useful method for isolation of an enzyme from many other contaminants. But in the case of β-galactosidase any adsorbent that had ever been tried was found to be nonspecific as regards the structure of glycoside moiety [3,4,8]. It was shown that lactose-coupled polyacrylamide gel was biospecific for β-galactosidase. This adsorbent will be useful for the separation of β-galactosidase from other sources because of its specificity and the mildness of the elution system. Although the form of linkage between lactose and polyacrylamide-hydrazide is not clear, it might be reasonable to consider the formation of a hydrazone. The fact that about 50% of β-galactosidase activity could not bind to this resin suggests that wheat-germ has two kinds of β-galactosidases, one of which is adsorbed and the other is not. The latter β-galactosidase might have no affinity for or is not sterically accessible to the ligand.

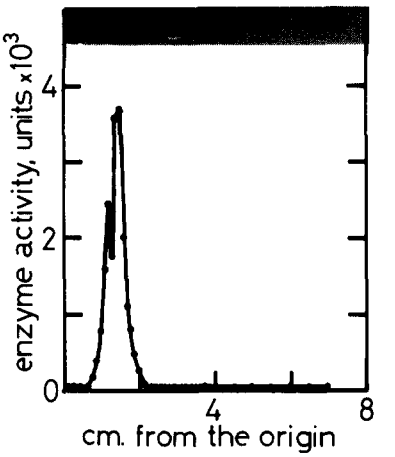

Fig.3. Polyacrylamide gel electrophoresis of wheat-germ β-galactosidase. About 20 μg each of protein sample was applied and after electrophoresis one column of gel was stained for protein. The other was sliced into 1 mm sections to detect enzyme activity.

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References

[1] Cuatrecasas, P. and Anfinsen, C. B. (1971) in: Annual Review of Biochemistry (Snell, E. E. ed) Vol. 40, pp. 259–278, Annual Reviews Inc., California.
[2] Steers, E., Cuatrecasas, P. and Pollard, H. B. (1971) J. Biol. Chem. 246, 196–200.
[3] O'Carra, P., Barry, S. and Griffin, T. (1974) in: Methods in Enzymology (Jakoby, W. B. and Wilchek, M. eds) Vol. 34, pp. 108–126, Academic Press, New York and London.
[4] Junowicz, E. and Paris, J. E. (1973) Biochim. Biophys. Acta 321, 234–245.
[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[6] Inman, J. K. and Dintzis, H. M. (1969) Biochemistry 8, 4074–4082.
[7] Davis, B. J. (1964) Ann. NY Acad. Sci. 121, 404–427.
[8] Mega, T. and Matsushima, Y. (1976) J. Biochem. (Tokyo) 79, 185–194.