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Note

Rapid isolation of *Escherichia coli* β-galactosidase by fast protein liquid chromatography

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The isolation of *Escherichia coli* β-galactosidase has received considerable attention during the last decade due to the various applications of this enzyme[1]. Traditional methods of isolation include several convenient chromatographic steps[2–4] or centrifugation in a sucrose gradient[5], which made the whole procedure laborious and time-consuming and reduced either the yield of enzyme, or its activity. This work presents a rapid, simple and efficient procedure for the isolation of *E. coli* β-galactosidase which uses the advantages of high-performance liquid chromatography (HPLC) of proteins.

EXPERIMENTAL

A fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) was used, consisting of two P-500 pumps, an injection valve MV-7 with 10-ml Superloop for sample injection, an LCC-500 chromatography controller, UV-monitor UV-1 with HR-10 flow cell (optical path 10 mm), equipped for detection at 280 nm, a fraction collector FRAC-100 and a REC-482 dual pen recorder. Prepacked chromatography columns of Mono Q HR 5/5 (5 cm × 5 mm I.D.) and Superose 6 HR 10/30 (30 cm × 10 mm I.D.) (Pharmacia) were used for ion exchange and gel filtration, respectively.

Reagents

Triethanolamine hydrochloride (TEA-HCl), phenylmethylsulphonyl fluoride (PMSF), tris(hydroxymethyl)aminomethane (Tris) and sodium dodecyl sulphate (SDS) were obtained from Serva, morpholinoethanesulphonic acid (MES), dithiothreitol (DTT) from Sigma, β-mercaptoethanol, Coomassie Blue G-250 from Loba Chemie and membrane filters XM-100 from Amicon; other reagents and salts were of analytical purity.

Protein in the crude extracts and ammonium sulphate fraction was assayed by the method of Bradford[6], and subsequently by UV-absorbance. The extinction of a 0.1% solution of β-galactosidase at 280 nm is 2.09[4].

Enzyme activity was assayed by the increase in absorbance at 420 nm in a mixture containing 2 m*M* *o*-nitrophenyl-β-D-galactopyranoside in 10 m*M* TEA-HCl buffer pH 7.4 with 1 m*M* magnesium chloride and 100 m*M* sodium chloride, incubated with enzyme at 37°C.

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SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli[7] in 8% gels with a Pharmacia GE-4 electrophoresis apparatus and an EPS 400/500 power supply. Gels were stained with 0.04% Coomassie Blue G-250 in 3.5% perchloric acid.

Isolation of the enzyme

Cells of *E. coli* ML 308 were grown in the liquid medium M63[8] with 0.5% glycerol and harvested in the middle of the log-phase. A 1-g amount of the cells was suspended in 10 ml of 10 m*M* Tris–HCl buffer pH 7.2 with 50 m*M* sodium chloride, 1 m*M* magnesium chloride, 2 m*M* β-mercaptoethanol and 0.1 m*M* PMSF, cooled in an ice-bath and sonicated three times for 4 min in a 15-kHz ultrasonic disintegrator until no intact cells were detected by microscopy. Cell debris was removed by centrifugation at 15 000 *g* for 10 min. To the crude extract obtained, finely powdered ammonium sulphate was slowly added to 40% saturation at +4°C with constant stirring. After 2 h the precipitate formed was collected by centrifugation at 10 000 *g* for 15 min and dissolved in 50 m*M* MES–sodium hydroxide buffer pH 6.0 with 1 m*M* magnesium chloride and 1 m*M* DTT (buffer A).

This solution was desalted at room temperature by passage through a Pharmacia PD 10 prepacked column equilibrated with buffer A. A portion of the desalted solution, containing 20–25 mg of protein, was applied to the Mono Q HR 5/5 ion-exchange column, equilibrated with the same buffer. The enzyme was eluted as an individual peak in a volume of 1.4 ml by a stepwise gradient of sodium chloride in buffer A at flow-rate of 1 ml/min. The threshold level for automated peak collection was 0.1 absorbance units.

The β-galactosidase peaks from Mono Q were collected, concentrated *ca.* 1 mg/ml protein by ultrafiltration on an XM-100 membrane filter and applied to the Superose 6 10/30 high-performance gel filtration column for final purification. The major protein peak was collected automatically with a threshold level of 0.05 a.u., concentrated on XM-100 in the same way, then glycerol was added to 50% and the enzyme was stored at −12°C.

RESULTS AND DISCUSSION

Since ion-exchange chromatography on Mono Q provides the major purification step in the described procedure (Table I), the pH and gradient shape were optimized to give the best resolution of the β-galactosidase peak. At pH 7.0 and 8.0

TABLE I

PURIFICATION OF β-GALACTOSIDASE FROM *E. COLI*

Fraction	*Total protein (mg)*	*Specific activity (U/mg)*	*Total activity (U)*	*Yield (%)*
Crude extract	150	15.3	2300	100
Ammonium sulphate, 0–40%	54	35.4	1900	95.7
Mono Q	2.1	587	1230	61.7
Superose 6	1.3	620	1180	59

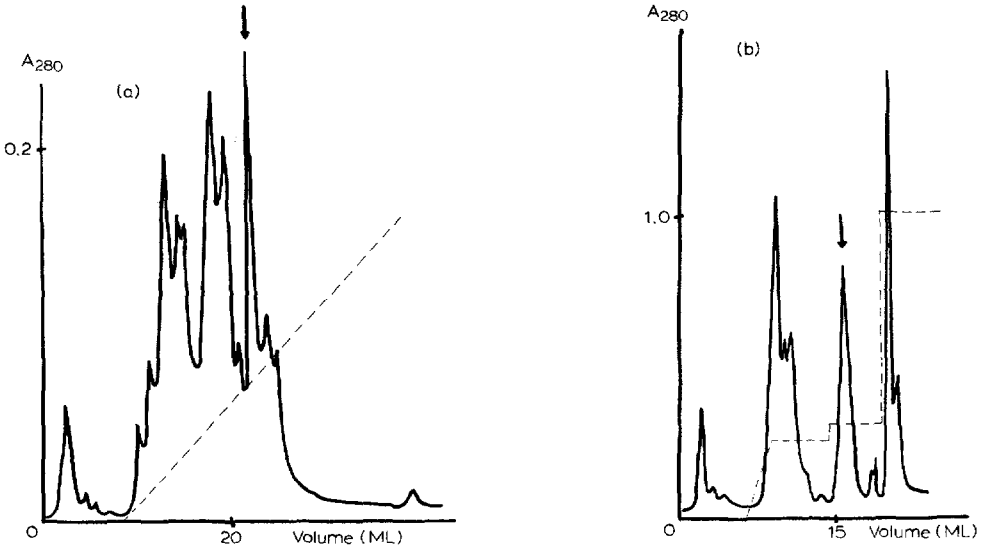

Fig. 1. Separation of *E. coli* β-galactosidase after ammonium sulphate precipitation on the Mono Q HR 5/5 column: (a) linear gradient 0–1 *M* sodium chloride in 30 ml of buffer A (see Experimental); (b) stepwise gradient of sodium chloride 0–0.25 *M* in 3 ml, 0.25 *M* in 6 ml, 0.3 *M* in 7 ml, 1 *M* in 6 ml of the same buffer. The salt concentration is given by the dashed line. Flow-rate 1.0 ml/min. Arrows indicate the β-galactosidase peaks.

the enzyme was bound too tightly to the exchanger and eluted only at high salt concentrations as a broad poorly resolved peak. Better separations were observed with the zwitterionic buffer MES–sodium hydroxide at pH 6.0. Under these conditions the enzyme was eluted as a narrow peak at *ca.* 0.3 *M* sodium chloride (Fig. 1a). A stepwise gradient (Fig. 1b) gave a better separation of the β-galactosidase peak from neighbouring peaks. The Mono Q step with a protein load of 20–25 mg for the

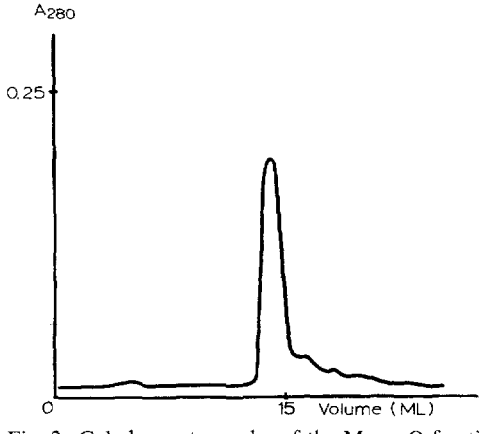

Fig. 2. Gel chromatography of the Mono Q fraction on the Superose 6 HR 10/30 column. Conditions: 50 m*M* TEA-HCl buffer pH 7.2, 50 m*M* sodium chloride, 1 m*M* magnesium chloride, 1 m*M* DTT; flow-rate 0.7 ml/min.

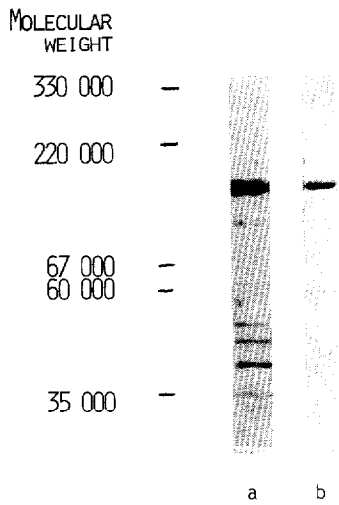

Fig. 3. SDS-PAGE of β-galactosidase: (a) Mono Q fractions, 9 μg; (b) Superose 6 fraction, 3 μg.

5 cm × 0.5 cm column took only 20 min and resulted in *ca.* 1 mg of enzyme, which had 95% purity according to SDS-PAGE. This enzyme can be used without further purification for many practical purposes.

When an higher purity is necessary, protein impurities can be efficiently removed by gel filtration on Superose 6 (Fig. 2). Any suitable buffer with pH 6–8 could be used with the salt concentration not less then 50 m*M* to reduce non-specific adsorption and 1 m*M* magnesium chloride and DTT to stabilize the enzyme. Gel filtration gives β-galactosidase with a specific activity of 620 U/mg which is homogeneous according to SDS-PAGE (Fig. 3). The total yield of the enzyme is 60% (Table I).

CONCLUSION

The procedure developed uses the advantages of the Pharmacia Mono Beads ion exchanger Mono Q and the FPLC system and takes 1 working day to get highly active homogeneous *E. coli* β-galactosidase in good yield starting from bacterial biomass. Analytical scale Mono Q HR 5/5 columns gave good results also on a semi-preparative scale. Further scaling-up with Pharmacia Mono Q HR 16/10 and preparative grade Superose HR 16/50 columns can easily provide tens of milligrams of homogeneous enzyme within the same time.

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