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Note

Purification of enzymes by medium-performance gel filtration on TSK-Gel Toyopearl

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Gel filtration of proteins at low speed on soft gels such as Sephadex has been applied for some time. However, high-performance gel filtration has recently become more important, especially in analytical separations. Also, medium-performance gel filtration has been applied recently. Medium-performance gel filtration may become very popular in the future for preparative separations[1], and some studies on separations of proteins[2], dextran[3], etc. have already been reported. However, more basic data must be accumulated for medium-performance gel filtration to be adopted extensively.

We have investigated the separation of enzymes by medium-performance gel filtration on TSK-Gel Toyopearl (Toyo Soda, Tokyo, Japan), which is the same material as Fractogel TSK available from E. Merck (Darmstadt, G.F.R.). The results on the recovery and the degree of purification are reported in this note.

EXPERIMENTAL AND RESULTS

Crude β-galactosidase from cultured bacteria[4] and commercial urease from jack bean (P-L Biochemicals, Milwaukee, WI, U.S.A.) were separated on Toyopearl HW55F of 30–60 μm particle size. The separation range of this support is reported to be mol.wt. 10,000–2,000,000 for globular proteins[2]. Two glass columns of 55 × 2.5 cm I.D. were used in series. The columns were made in our laboratory and have end-fittings on both ends to make dead-space volume minimal. The columns were packed by the constant-velocity method[5] with a peristaltic pump (Model SJ-1211H; Atto, Tokyo, Japan). The same peristaltic pump was used to deliver eluent, and a valve injector (Model 7010; Rheodyne, Berkeley, CA, U.S.A.) was used for sample injection. The column effluent was continuously monitored for total protein concentration with a UV detector (Model SF-770; Schoeffel, Westwood, NJ, U.S.A.) at a wavelength of 280 nm and then collected in 5-ml fractions with a fraction collector (Model SF-100G; Toyo Kagaku, Tokyo, Japan). The fractions were examined for β-galactosidase activity with *o*-nitrophenyl-β-D-galactopyranoside or for urease activity by the colorimetric timing method[6]. The eluent was 0.2 *M* phosphate buffer, pH 6.7. The flow-rate was 1 ml/min. The separations were carried out at 22°C. Samples of 45 mg in 3 ml eluent were injected.

Fig. 1 shows the result of separation of crude β-galactosidase. The yield of

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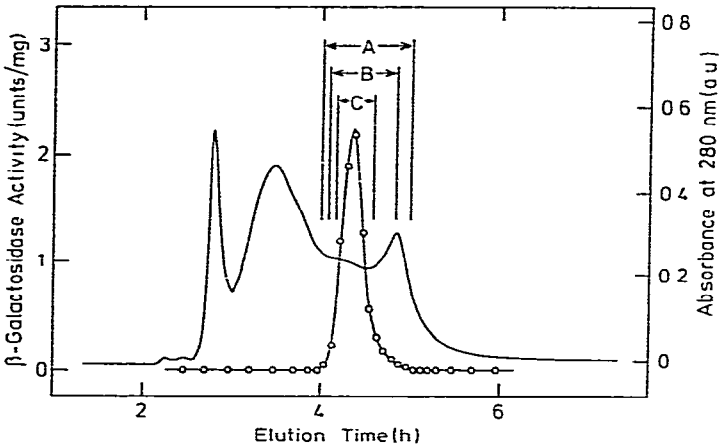

Fig. 1. Purification of crude β-galactosidase on Toyopearl HW55F. ——, Distribution of total proteins monitored by UV absorbance at 280 nm; O — O, distribution of β-galactosidase monitored by enzymatic activity.

enzymatic activity, specific activity and degree of purification are summarized in Table I for cases in which 12 fractions (A: all fractions containing β-galactosidase activity), 9 fractions (B) and 5 fractions (C) were combined together. Fig. 2 shows the result of separation of commercial urease. The yield of enzymatic activity, specific activity and degree of purification are summarized in Table II for cases in which 20 fractions (A: all fractions containing urease activity) and 13 fractions (B) were combined together. Enzymatic activities were recovered almost quantitatively for both samples. Moreover, a fairly high degree of purification was achieved with high yield. Most impurities have lower molecular weights than urease, especially in the case of commercial urease, and therefore the impurities and urease were separated well. As a result, specific activity increased 10-fold with a yield higher than 90%. Besides, the separation on Toyopearl is considerably rapid compared with the separation on conventional soft gels. Accordingly, Toyopearl seems to be an ideal support for medium-performance gel filtration.

TABLE I

PURIFICATION OF CRUDE β-GALACTOSIDASE ON TOYOPEARL HW55F

Fractions A, B and C: see text and Fig. 1.

Fraction	*Yield (%)*	*Specific activity (units/mg)*	*Degree of purification*
Original sample		0.95	
A	94	2.8	2.9-fold
B	93	3.7	3.9-fold
C	83	6.4	6.7-fold

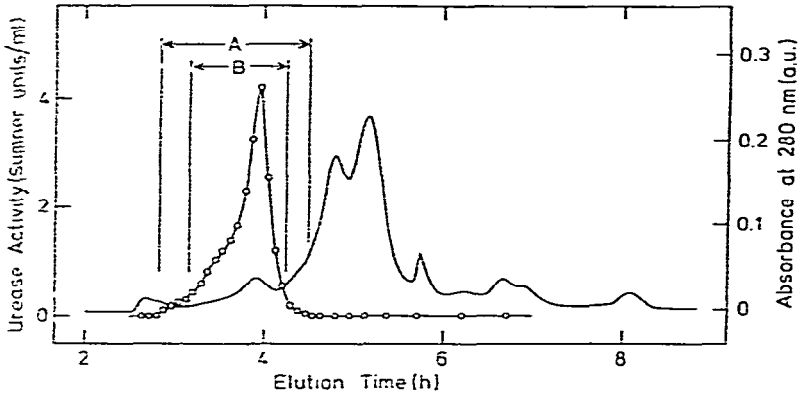

Fig. 2. Purification of commercial urease on Toyopearl HW55F. ——, Distribution of total proteins monitored by UV absorbance at 280 nm; O—O, distribution of urease monitored by enzymatic activity.

TABLE II

PURIFICATION OF COMMERCIAL UREASE ON TOYOPEARL HW55F

Fractions A and B: see text and Fig. 2.

Fraction	*Yield (%)*	*Specific activity (units/mg)*	*Degree of purification*
Original sample		2.5	
A	98	17.0	6.8-fold
B	92	25.6	10.2-fold

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