The Isolation of Invertase from Baker's Yeast: A Four-Part Exercise in Protein Purification and Characterization

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These exercises evolved from modifications to the one-step purification of yeast invertase (E.C. 3.2.1.26) described by Melius *(1)* and are intended to (i) exploit properties that make invertase a logistically attractive target of a purification project for the large and diverse enrollments of a one-semester course in biochemistry; (ii) obtain a final product that is comparable to a commercial standard of pure invertase; and (iii) enhance a student's ability to "visualize" and, therefore, understand the process by which a colorless enzyme is selectively detected and separated from other proteins in a mixture.

The isolation of invertase offers several advantages for a protein purification project for undergraduate students. First, the starting material (dried baker's yeast) is inexpensive and readily available from most local grocery stores. Second, the enzyme is easily extracted from the periplasmic space by overnight autolysis in a bicarbonate solution *(1)*. Third, and most important for a sequential purification project, the enzyme is remarkably stable (no measurable loss of activity is observed in yeast extracts stored for up to 5 weeks at 4 °C). Finally, the substrate of the activity assay (sucrose) is readily available and the orange-colored product of the "stop-assay" (3-amino-5-nitrosalicylate) can be monitored with any visible spectrophotometer.

In contrast to the one-step partial purification method first described by Melius *(1)* and later characterized by Roberts et al. *(2)*, the purity of invertase isolated in this three-step procedure is much more comparable to invertase purchased from Sigma Chemical Company *(3)* as judged by both SDS–PAGE analysis and specific activity measurements. This project can be incorporated into the curriculum of any biochemistry laboratory supplied with (i) a refrigerated, high-speed centrifuge equipped with both preparative- and standard-sized rotors; (ii) visible spectrophotometers; (iii) gel filtration columns equipped with flow adaptors and peristaltic pumps; (iv) casting frames and plates for pouring mini-slab gels; and (v) an electrophoresis power supply. While this four-week exercise requires substantial instructor preparation time (preparing yeast extract and other solutions, packing chromatography columns, and maintaining pumps and electrophoresis equipment), the schedule can be easily extended, if desired, to allow students to gain valuable experience with these routine tasks.

Because the enzyme is colorless, invertase-enriched fractions must be detected indirectly through activity measurements. For this reason, the sequence of steps was designed to maximally visualize the isolation process. For example, the large protein pellet precipitated in 29% ethanol is discarded after it is shown to be essentially devoid of invertase activity while the smaller, invertase-enriched pellet precipitated between 29–40% ethanol is saved. In addition, the 29–40% ethanol cut is spiked with colored size markers (blue dextran and hemoglobin) that elute from the gel filtration column immediately before and after the invertase-enriched fractions (Figure 1). Finally, the purity of samples reserved from each stage of the separation are visually assessed by SDS–PAGE analysis (Figure 2) before the more quantitative (but less intuitive) specific activity results are calculated and summarized in a purification table.

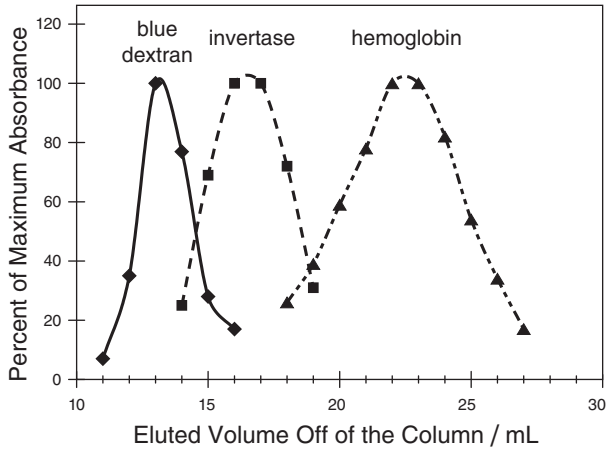

Figure 1. Elution profiles of blue dextran, invertase, and hemoglobin from a column of Sephacryl HR300. The blue dextran fractions (11–16) were detected on a Novaspec spectrophotometer at 620 nm. The red-tinted hemoglobin fractions (18–27) were detected at 420 nm. Invertase activity in 10.0 μL samples of fractions 14–19 was monitored by the reduction of 3,5-dinitrosalicylate by glucose to produce the orange-colored product (3-amino-5-nitrosalicylate), which is detected at 540 nm *(1, 6)*.

Overview and Summary

Week 1

Yeast extracts are prepared by overnight autolysis in 0.10 M sodium bicarbonate at 35 °C as described by Melius *(1)*. We prefer the bread machine varieties of baker's yeast that contain significantly greater activity than active dry yeast (Table 1). The project is initiated by isolating invertase from a 25.0 mL sample of yeast extract by differential precipitation in ethanol solutions (fraction 1, F1, Figure 2). The invertase that precipitates between 29–40% ethanol is suspended (and concentrated relative to the initial 25 mL sample) in 2.0 mL of gel filtration column buffer (fraction 2, F2).

Week 2

One-half (i.e., 1.0 mL) of the 29–40% ethanol cut (F2) is purified by gel filtration chromatography on a 40–45 cm × 1 cm column packed with Sephacryl HR-300 resin (Figure 1). The four 1 mL fractions containing the most invertase activity are pooled (fraction 3, F3) then purified and concentrated by anion-exchange chromatography (F4, fraction 4) on a ½ mL bed volume of diethylaminoethyl (DEAE) cellulose.

Table 1. Comparison of Invertase Activity and Total Protein from Different Sources of Baker's Yeast

Source of Extract[a]	Invertase Activity[b] (units per 25 mL extract)	Total Protein[c] (mg per 25 mL extract)	Specific Activity/ (units/mg)
Fleischmann's Active Dry Yeast (n = 4)	1500 (1070–1980)	120 (33.0–200)	27 (9.2–60)
Fleischmann's Bread Machine Yeast (n=4)	2610 (2160–3370)	163 (52.8–262)	14 (8.8–25)
Red Star Active Dry Yeast (n = 3)	486 (421–535)	280 (205–373)	1.8 (1.4–2.4)
Red Star Bread Machine Yeast (n = 3)	6260 (4700–7600)	255 (150–354)	25.9 (21–31)

[a]Each extract was prepared by overnight (about 15 hours) autolysis in 0.10 M $NaHCO_3$ at a concentration of one gram of dried yeast per 3.6 mL of bicarbonate solution at a temperature of 35 °C. Following centrifugation at 15,000x g for 30 min at 4 °C, each extract was poured off the pellet of insoluble debris and stored at 4 °C. [b]One unit of invertase activity is defined as the hydrolysis of 1 μmole of sucrose per minute at pH 4.8, 20 mM initial sucrose concentration, and ambient laboratory temperature (about 20 °C). Results represent the average and range of values (in parenthesis) measured from extracts prepared from different lots (n) of each source. [c]Proteins were measured by a modification of the Bradford dye binding method *(7)* using bovine serum albumin as the reference protein.

Weeks 3 and 4

Samples reserved from each of the four fractions (F1–F4) are visually assessed and compared by SDS–PAGE analysis (week 3) against a commercial sample of invertase purchased from Sigma Chemical Company (Σ) (Figure 2) then quantitatively assessed (week 4) by specific activity measurements and summarized in a purification table (Table 2).

Procedures

Details regarding the purification procedures, SDS–PAGE analysis, and the measurement of invertase activity and total protein are provided as online material.

Hazards

Acrylamide is a neurotoxin and is readily absorbed through skin. Inhalation may be fatal. Sodium azide is a poison. It may be fatal if swallowed or absorbed through skin. 3,5-Dinitrosalicylic acid is harmful if swallowed or absorbed through the skin. β-Mercaptoethanol may be fatal if absorbed through skin or inhaled. Sodium hydroxide, hydrochloric acid, and phosphoric acid are corrosive and may cause burns. Students should wear gloves and avoid direct contact with the solutions.

Results of SDS–PAGE Analysis

The commercial sample of pure yeast invertase (the Σ labeled lane in Figure 2) is detected in Coomassie blue stained SDS–PAGE gels as a smeared band that migrates between the 100 and 150 kDa size markers (the lane labeled M). These results agree with previous reports describing invertase as a homo-dimer with a native mass of about 270 kDa *(4, 5)*. The "smeared" appearance of the invertase band (a smudge compared to the tight bands of other polypeptides) is due to the sugars that account for nearly 50% of the enzyme's total mass *(5)*. In some ways, this unusual band is a benefit in that it provides a "finger-print" by which the invertase band can be distinguished from other proteins in the gel.

One of the most apparent conclusions derived from the gel (Figure 2) is that the final fraction isolated by our students (F4) is typically not as pure as the commercial invertase (Σ) as judged by both the greater number and relative intensity of contaminating bands observed in the lanes labeled F4 versus Σ. On the other hand, the gel unambiguously demonstrates the sequential isolation of invertase from the initial sample of yeast extract (F1) to the final fraction (F4). That is, the *relative intensity* of the

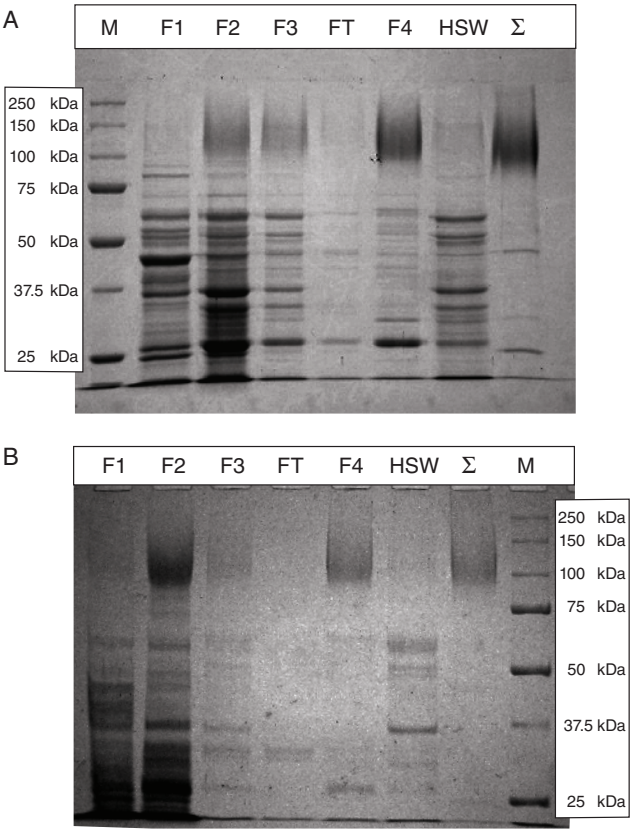

Figure 2. SDS–PAGE analysis of yeast invertase fractions on discontinuous (A) versus continuous (B) polyacrylamide slab gels. Lane labels: F1 = yeast extract; F2 = 29–40% ethanol cut; F3 = gel filtration peak; FT = DEAE flow through; F4 = DEAE-enriched invertase; HSW = high salt wash from DEAE column; Σ = commercial invertase sample (Sigma Chemical Company); M = Biorad Precision Plus Molecular Weight Markers.

Table 2. Purification Table for the Isolation of Invertase from Baker's Yeast[a]

Fraction	Volume/mL	Total Protein[b]/mg	Total Activity[c]/units	Specific Activity/ (units/mg)	*n*-Fold Purification (vs F1)	Activity Yield (%) (vs F1)
F1	25.0	39 ± 6	1070 ± 95	28 ± 5	1.0	100
F2	2.0	8.2 ± 2.6	717 ± 100	94 ± 24	3.4	67
F3	4.0	0.72 ± 0.33	145 ± 60	210 ± 80	7.5	13.5[d]
F4	1.0	0.16 ± 0.08	72 ± 27	490 ± 180	17.5	6.8[e]
Σ	1.0	0.11 ± 6	115 ± 15	1100 ± 300	39	---

[a]All data reflect the average values and standard deviation reported by 8 groups of students from two laboratory sessions. [b]Protein values were measured by a modification of the Bradford dye binding method *(7)* using bovine serum albumin as reference. [c]Invertase activity was monitored by the reduction of 3,5-dinitrosalicylate by glucose to produce the orange-colored product (3-amino-5nitrosalicylate) that is detected at 540 nm *(1, 6)*. [d]40% of F2 loaded onto size column. [e]50% of F3 loaded onto DEAE column. F1= yeast extract; F2 = 29–40% ethanol cut; F3 = gel filtration peak; F4 = DEAE-enriched invertase; Σ = commercial invertase dissolved in gel filtration column buffer to a final concentration of 1 mg per mL.

invertase band (the characteristic smudge) compared to that of other contaminating polypeptides progressively increases with each fraction from F1 to F4.

Results of Specific Activity Measurements

The incremental increases in specific activity for the same fractions (F1–F4) previously analyzed by SDS–PAGE are shown in Table 2. In agreement with the results of the gel, the commercial enzyme (Σ) has a higher specific activity (1100 units per mg) and is, by definition, about two times more pure than the DEAE-enriched fraction of invertase (F4, 490 units per mg) typically isolated by our students. That is, the reduced intensity of contaminating bands observed in the gel for the commercial enzyme corresponds to a higher specific activity value because the commercial sample contains more enzyme activity but less total protein.

The mass of invertase contained in the samples of the different commercial yeast extracts (Table 1) can be estimated by dividing the total number of units of invertase activity in the initial 25 mL sample of yeast extract (400–8000 units) by the specific activity value of the pure, commercial enzyme (1100 units per mg). A range of 0.4 to 7 mg is obtained. The nature of the challenge that a student confronts in the isolation of a single protein from a biological mixture is now apparent by noting that the initial yeast extracts contain hundreds of milligrams of total protein but only 0.4 to 7 mg of invertase.

In the first step of the procedure (differential precipitation in ethanol solutions), the recovery of 65–70% of the initial activity but only 20% of the total protein in the 29–40% ethanol cut (F2) translates to a *3.4-fold increase* in the specific activity or purity of the enzyme (3.4 times as much enzyme activity was recovered compared to total protein). The precipitation procedure, in terms of specific activity increase, is typically the best step in the purification procedure. The peak fractions from the gel filtration column (second step of the procedure) yields about 40% of the activity versus 17% of the total protein applied to the column for an additional *2.2-fold increase* in purity or specific activity of the enzyme (and a combined *7.5-fold increase* in purity over the initial extract). The last step of the procedure (anion-exchange chromatography) yields about 50% of the applied activity versus 22% of the total protein for a final *2.3-fold increase* in specific activity (and a net *17.5-fold increase* in purity over the initial yeast extract).

While the yield of initial activity from F1 recovered in the final sample (F4) is only 6.8% (Table 2), the data do not reflect that only one-half of the 29–40% ethanol cut (F2) was applied to the gel filtration column. Taking this into account provides an adjusted yield of about 15% of the initial activity for the three-step procedure.

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Abstract and keywords

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Supplement

Details regarding the purification procedures, SDS–PAGE analysis, and the measurement of invertase activity and total protein

List of reagents and solutions

Protein purification and characterization handout

Student handouts