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Note

Affinity purification of dextransucrase from *Streptococcus sanguis* ATCC 10558*

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Dextransucrase (EC 2.4.1.5) is an exocellular glucosyl transferase produced by various species of *Leuconostoc* and *Streptococcus*[1,2]. This enzyme, which appears to play an important role in the formation of dental caries[3,4], catalyzes the transfer of D-glucose from sucrose to a D-glucan (dextran) with concomitant release of fructose[5]. Dextransucrase from *Streptococcus sanguis* exists as an aggregate ($M_r > 5 \times 10^6$) that is dissociated into inactive monomeric forms (mol. wts. 174 000 and 156 000) in the presence of sodium dodecyl sulfate[6,7]. Restoration of enzymatic activity is achieved by the addtion of a nonionic detergent (such as Triton X-100 or octyl β-D-glucopyranoside) to SDS-inactivated dextransucrase[8,9].

Dextransucrase binds dextrans of varying composition[10], a fact which led investigators to use Sephadex gels as affinity columns for the purification of the enzyme from *Leuconostoc mesenteroides*[11] and *Streptococcus mutans*[12]. Elution was achieved using solutions of urea or guanidine HCl (which required extensive dialysis for removal) or dextran (which was removed by treatment with dextranase). We have noted[13] that, in order to study the multiple reactions of dextransucrase, the enzyme must be free of sugars. Thus the presence of dextran in these preparations may compromise experimental results, since the degree of removal of this acceptor sugar is uncertain. We report herein a rapid and simple affinity-based purification that utilizes the ability of SDS to elute enzyme bound to Sephadex G-200. This initial step yields carbohydrate-free dextransucrase (>80% homogeneous) from a crude mixture. The enzyme is obtained free of detergents and with >95% purity by use of two hydroxyapatite chromatography steps.

RESULTS AND DISCUSSION

Crude concentrated enzyme was chromatographed on a Sephadex G-200 column (Fig. 1). The bulk of the contaminating proteins (peak A) washed through the column,

* Abbreviations used: DS, dextransucrase; HA, hydroxyapatite; PAS, periodic acid–Schiff's base; SA, specific activity; SDS, sodium dodecyl sulfate; SDS–PAGE, SDS–polyacrylamide gel electrophoresis.

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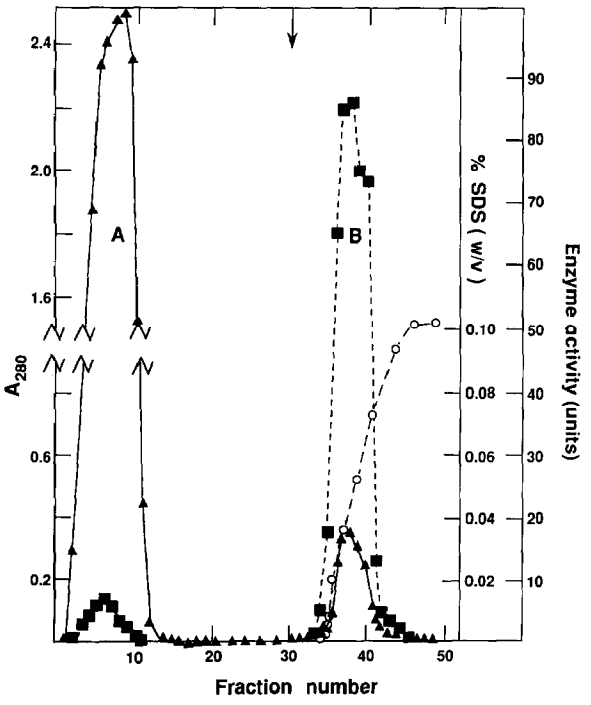

Fig. 1. Affinity purification of dextransucrase on Sephadex G-200. [Crude concentrated dextransucrase (50 mL, 300 units) was applied to a 2.5 × 15 cm column of Sephadex G-200 and washed with about 200 mL of equilibration buffer (10mM sodium phosphate, pH 6.4, 0.02% NaN_3). Enzyme was eluted (arrow) with equilibration buffer containing 0.1% SDS. Fractions were collected and analyzed for enzyme activity (■), u.v. absorbance (▲), SDS concentration (○), and protein (not shown)].

while the majority of dextransucrase remained adsorbed. About 15% of the applied enzyme failed to bind to the column; reapplication of this fraction to an identical Sephadex G-200 column resulted in the same relative adsorption and elution profile. The reason for this behavior is unclear, but the results indicate that column overload is not the cause. SDS (0.1%) eluted approximately 60% of the applied enzyme (peak B) which was recovered with an avarage specific activity of 67 units.mg^{-1}. The average concentration of SDS in the pooled fractions was 0.05% (w/v), and no sugar was present in the peak B pool. The Sephadex G-200 column can be reused indefinitely after extensive washing with detergent-free buffer.

SDS–PAGE analysis of enzyme samples, followed by Coomassie staining (Fig. 2B, lane 3), showed one major contaminant. The silver-stained gel (Fig. 2A, lane 3) indicated several faint bands which may arise from components of the growth medium. The major contaminant (mol. wt. 45 000) may be a glucan-binding protein[14]. The activity gel (Fig. 2C) indicated two enzyme forms — the nascent mol. wt. 174 000 protein and a mol. wt. 156 000 polypeptide derived from the former by proteolytic cleavage[7].

Peak B from the Sephadex G-200 column was subjected to chromatography on hydroxyapatite (Fig. 3) in the presence of SDS. This step effectively removed the mol.

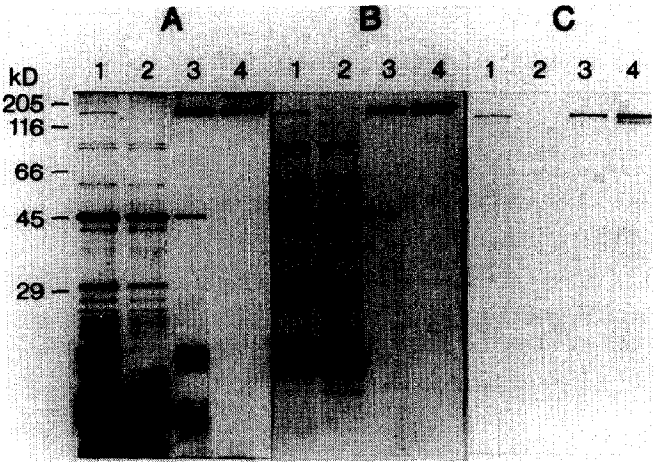

Fig. 2. SDS–PAGE analysis of various purification steps. [Samples were prepared by lyophilization of pooled preparations; the lyophils were dissolved in sample buffer (60mM Tris, pH 6.8, 5% 2-mercaptoethanol, 2.3% SDS, 10% glycerol, and bromophenol blue tracking dye). Aliquots containing 3–20 μg of protein (for Coomassie staining; 0.15–1 μg for silver strain or activity determination) were applied to 3% stacking–12% resolving gels. Electrophoresis was run at 20–25 mA constant current at 4°, and the gels were stained with Coomassie dye, silver, or PAS stain (see Experimental). Lane 1, crude enzyme; lane 2, peak A from Sephadex G-200 column; lane 3, peak B from Sephadex G-200 step; lane 4, enzyme peak from SDS–HA chromatography step].

wt. 45 000 protein and the minor contaminants from dextransucrase (Figs. 2A and 2B, lane 4). Based on activity determinations, complete recovery of the enzyme (specific activity of 75 units.mg^{-1}) was achieved. The SDS-inactivated dextransucrase was reactivated by incubation with nonionic detergent, and the enzyme was recovered by chromatography on hydroxyapatite. This final step yielded detergent-free dextransucrase without loss of activity or protein. Table I summarizes the relevant data for this purification protocol.

EXPERIMENTAL

Purification of dextransucrase. — Lyophilized cultures of *Streptococcus sanguis* ATCC 10558 were grown in 12 L of brain–heart infusion medium (Difco Laboratories, Detroit, MI) ultrafiltrate (< 10 000 mol. wt.) supplemented with glucose as the primary carbon source. Cells were removed by centrifugation, and the supernatant fluid was dialyzed and concentrated using an Amicon H1P 100-20 hollow-fiber device. The concentrated enzyme solution was dripped into liquid nitrogen to form frozen pellets which were stored at −70°. All chromatographic procedures were run at 25°.

Enzyme (approximately 300 units) was applied to a column (2.5 × 15 cm) of Sephadex G-200 (40–120 μm; Pharmacia Fine Chemicals, Piscataway, NJ) and washed with about 200 mL of 10mM sodium phosphate, pH 6.4, containing 0.02% NaN_3. The column was washed with the same buffer containing 0.1% SDS to elute the enzyme. This enzyme was pooled and either frozen in liquid nitrogen (and stored at −70° until used)

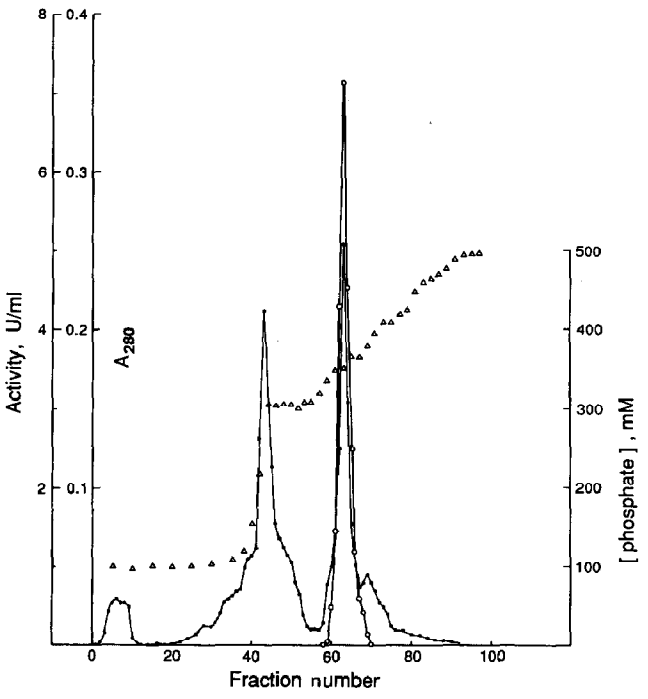

Fig. 3. Chromatography of Sephadex G-200 peak B on hydroxyapatite. [Peak B from the Sephadex G-200 column was brought to 0.1% SDS (w/v) and applied to a column (1.7 × 7 cm) of hydroxyapatite that had been equilibrated in 100mM sodium phosphate, pH 6.3, 0.1% SDS, 0.02% NaN_3. The column was washed with 50 mL of this buffer, then with 50 mL of 300mM sodium phosphate, pH 6.3, 0.1% SDS, 0.02% NaN_3. Dextransucrase was eluted by application of a linear gradient (120 mL) of 300mM to 500mM sodium phosphate, pH 6.3, containing 0.1% SDS and 0.02% NaN_3. Fractions were assayed for activity (○), u.v. absorbance (■), protein (not shown), and conductivity (△), which was compared to a standard curve generated from the sodium phosphate buffers containing 0.1% SDS and 0.02% NaN_3].

or brought to 0.1% SDS and applied to a column (1.7 × 7 cm) of hydroxyapatite (Bio-Gel HTP; Bio-Rad Laboratories, Richmond, CA) that was equilibrated in 100mM sodium phosphate (all buffers for SDS–HA chromatography were pH 6.4 and contained 0.1% SDS and 0.02% NaN_3). The column was washed with about 50 mL of equilibration buffer, then with 50 mL of 300mM sodium phosphate buffer solution. The enzyme was eluted by application of a linear gradient (120 mL) of 300mM to 500mM sodium phosphate buffer solution. Fractions containing enzyme activity were pooled, frozen in liquid nitrogen, and stored at −70°.

SDS-inactivated dextransucrase (about 50 units) from the SDS–hydroxyapatite chromatography step was dialyzed against water (2 × 2 L; 40 min) to reduce the phosphate concentration. This solution was brought to 2% (w/v) by addition of solid octyl β-D-glucopyranoside and incubated for 30 min at 25°. This mixture was applied to a column (0.7 × 7 cm) of hydroxyapatite equilibrated in 10mM sodium phosphate, pH 6.3. The bulk of the detergent was removed by washing the column with approximately 50 mL of equilibration buffer; dextransucrase was eluted by washing the column with

TABLE I

Summary of dextransucrase purification

Fraction	*Activity (units)*	*Protein (mg)*	*SA (U.mg^{-1})*[a]	*Recovery (%)*[b]
Concentrated crude[c]	355	33.7	10.5 (10.6)	—
Sephadex G-200[c]	215	3.2	67.1 (67.5)	60.5
SDS–HA[d,e]	158	2.1	75.2 (73.8)	99.6

[a] Specific activity is calculated from the average activity and protein. The numbers in parentheses are the averages. [b] The recovery of activity for each step. [c] Average of eight runs. [d] Average of six runs. [e] A portion of the Sephadex G-200 fraction was utilized in this step.

300mM sodium phosphate, pH 6.3. After dialysis against water (2 × 2 L; 30 min) the enzyme was frozen in liquid nitrogen and stored at −70°.

Analyses. — Dextransucrase activity was determined using a coupled enzyme assay system[7]. Aliquots of SDS-inactivated DS were reactivated by addition of an equal volume of 10% Triton X-100, followed by incubation for 20 min at 25°. This mixture was diluted with water to a final volume of 100 μL before addition of substrate. The concentration of SDS was determined by use of a dye-binding method[15]; the anthrone assay[16] was utilized in determination of sugar content. Protein was measured by absorbance at 280 nm or by the bicinchoninic acid protein assay[17]. SDS–PAGE was run as described by O'Farrell[18] using a 12% resolving gel–3% stacking gel system. The gels were stained with silver[18] or Coomassie brilliant blue. To detect active enzyme, duplicate gels were soaked in a solution of 5% sucrose, 1% Triton X-100, 0.02% sodium benzoate, pH 6.0, then stained for polysaccharide with periodic acid–Schiff's base[20].

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