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Cellular Association of Glucosyltransferases in *Leuconostoc mesenteroides* and Effects of Detergent on Cell Association

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Abstract

Most glucosyltransferase (GTF) activity in sucrose-grown cultures of some strains of *Leuconostoc mesenteroides* is found with the cell pellet after centrifugation. GTFs are known to bind to dextrans, and it was traditionally assumed that cell-associated GTFs were bound to those dextrans that cosedimented with the cells. We used a mutant strain (LC-17), derived from strain NRRL B-1355, which produced dextransucrase in the absence of dextrans, to investigate the extent to which GTFs were bound to cells or dextrans. Much of the GTF activity in glucose-grown cultures of strain LC-17, which do not produce dextran, was located in the cell pellets. Soluble enzyme activity increased when cell suspensions from glucose- or sucrose-grown cultures were incubated with mild nonionic detergents or zwitterionic reagents. Alternansucrase produced by the parent strain B-1355 was almost entirely associated with cells under conditions in which dextrans were or were not produced. Alternansucrase, but not dextransucrase, tended to be enriched in the particulate fraction of B-1355 cells that had been broken in a French press. The distribution of alternansucrase and the effects of detergents on the distribution of GTFs suggest that soluble GTFs sequestered in the cytoplasm, and GTFs bound or adsorbed to the cell membrane are probably the major contributors to the cell-associated GTF activity.

Index Entries: Dextransucrase; glucosyltransferase; *Leuconostoc mesenteroides*; solubilization; cell-associated enzyme; alternansucrase.

Introduction

Strains of *Leuconostoc mesenteroides* produce glucosyltransferases (GTFs), which synthesize dextrans (and other glucans) by transferring

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glucosyl units from sucrose to nascent carbohydrate chains: n sucrose → $(\text{glucose})_n + n$ fructose. GTFs and dextrans are normally produced in cultures grown on sucrose, but not in cultures grown on other sugars *(1)*. Dextransucrase (sucrose: 1,6-α-D-glucan 6-α-D-glucosyltransferase, EC 2.4.1.5), the best known GTF *(2,3)*, synthesizes dextrans, in which the primary chain consists mostly of chains of glucopyranose residues connected by α(1→6) glucosidic linkages. Different strains of *L. mesenteroides* produce dextrans with different structures *(1,2)*. Some dextrans exhibit varying degrees of branching by α(1→2), α(1→3), and α(1→4) glucosidic linkages. A few strains (B-1355, B-1501, B-1498) also produce alternansucrase (EC 2.4.1.140), which synthesizes alternan, a related neutral polysaccharide consisting of alternating α(1→6)- and α(1→3)-linked glucose units in the primary chain *(4,5)*. In general, it is assumed that a given type of GTF will synthesize a dextran of a given structure, although some variation in structure, depending on conditions under which the dextran is synthesized, has been reported for GTFs of strain B-1299 *(6,7)*. Some strains of *L. mesenteroides* produce one type of GTF and one type of dextran, whereas others produce multiple types of GTFs and dextrans (glucans). Differences in the proportions of types *(8)* are evident from both heterogeneity in glucan solubility profiles and electrophoretic band patterns of the GTFs. Besides being used to synthesize commercial dextrans for use as blood plasma extenders and molecular sieves *(1,3,9)*, GTFs are used to modify carbohydrates *(10)* and to synthesize novel sugars for use in the cosmetics industry *(11)*.

Distribution between the cell and supernatant fractions of GTF activities produced by *L. mesenteroides* in sucrose cultures varies over a wide range, depending on the strain *(8,12–14)*. GTFs are known to bind to dextrans, so it was traditionally assumed that the presence of cell-associated GTFs in the cell pellet after centrifugation of the cultures resulted from binding to high molecular weight dextrans that tended to pellet with the cells. The supernatant fractions, which are routinely used to monitor GTF production by cultures, were assumed to contain types of GTFs in the same proportion as in the cell fractions. Recent evidence *(6,15)* suggests that the supernatant fraction is not always a reliable indicator of GTF production. Moreover, since the proportion of total GTF activity associated with the cell fraction can approach 90% or more in sucrose cultures of some strains (*[8]*; also Smith and Zahnley, unpublished data), discarding the cell-associated GTFs can represent a substantial loss of activity during purification and may neglect GTFs capable of generating useful products. For strain B-1299, which produces 60–95% of cell-associated GTF *(12–14)*, some catalytic properties of the GTF activities in the soluble and insoluble fractions are similar (K_m values for sucrose and activation energies) but others differ, notably the pH optima for initial activity and the products formed *(6)*. This led Dols et al. *(6)* to suggest that synthesis of α(1→3) linkages (favored at low pH) could occur by a mechanism different from synthesis of α(1→6) and α(1→2) linkages.

The possibility that significant amounts of the cell-associated GTFs are actually associated with the cells rather than with dextrans produced by the cultures in sucrose medium has never been investigated. This distinction is critical to designing a strategy for improving the recoveries of GTFs by increasing the proportion of supernatant enzymes in the cultures. We have found *(15)* that most of the alternansucrase produced by *L. mesenteroides* strains B-1355 or B-1501 grown on glucose medium (treated to eliminate sucrose) is pelleted with the cell fraction, indicating that association of GTF activity with cells is not owing to binding by glucans; however, we did not examine subcellular distribution of GTFs. The purpose of the present study was to determine how the GTFs are localized and whether significant cell-associated GTF activity can be rendered soluble by treatment with nondenaturing or reversibly denaturing agents. To circumvent the problem of dextran binding by GTFs, we grew strains B-1355 and LC-17 (a mutant strain derived from B-1355) under conditions in which dextrans are not produced. The results concur with our other evidence *(15)* that the GTFs produced were mostly associated with the cells, even though dextrans are absent. We report herein on the differential distribution of GTFs in lysed cells and show that specific dissociating agents can be used to solubilize part of the cell-associated GTFs.

Materials and Methods

Bacterial Strains and Culture Media

Strain NRRL B-1355 was obtained from the National Center for Agricultural Utilization Research (NCAUR, USDA-ARS, Peoria, IL) stock culture collection. Strain LC-17 (NRRL 23182), a constitutive mutant that synthesizes high levels of GTFs in glucose medium, was derived from the ultraviolet mutant strain SL-1 *(16)* in our laboratory *(17)* by mutagenesis with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (ICN, Irvine, CA). Cultures were grown overnight at 30°C with shaking at 150 rpm on GTF medium buffered with 100 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES) as described previously *(16)*, except that glucose replaced sucrose where indicated.

Chemicals

Dextran (average mol wt 73,000), dinitrosalicylic acid (DNS), and reagents for sucrose phosphorylase assay were purchased from Sigma (St. Louis, MO). MES buffer was purchased from Research Organics (Cleveland, OH). Table 1 gives the sources of detergents and other potential dissociating agents. Other chemicals were reagent grade commercial products.

Assays of Enzyme Activity and Protein Content

GTF activity was determined by the release of reducing sugar, using the DNS assay as described previously *(16)*. One unit of GTF activity is defined as the amount of enzyme that liberates 1 µmol of reducing sugar/min

Table 1
Detergents and NDSBs Tested as Dissociating Agents

Name	Trivial name/ abbreviation	Source
Nonionic		
Isotridecylpoly(ethyleneglycolether)$_n$	$iC_{13}E_8$	Boehringer Mannheim, Indianapolis, IN
Octyl β-D-glucopyranoside	OG	Boehringer Mannheim; Calbiochem, La Jolla, CA
Octyl β-D-thioglucopyranoside	OSG	Calbiochem
Triton X-100	—	Sigma, St. Louis, MO
Zwitterionic		
3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate	CHAPS	Boehringer Mannheim; Calbiochem
N-decyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate	SB 3-10	Calbiochem
N-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate	SB 3-12	Calbiochem
N-tetradecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate	SB 3-14	Sigma
N-dodecyl-*N*,*N*-(dimethylammonio)butyrate	DDMAB	Calbiochem
N-dodecyl-*N*,*N*-dimethylglycine	Empigen BB	Calbiochem
N-ethyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate	NDSB195	Calbiochem
3-(1-pyridinio)-1-propanesulfonate (Synonym: 1-[3-sulfopropyl]pyridinium hydroxide, inner salt)	NDSB201	Aldrich, Milwaukee, WI
N-benzyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate	NDSB256	Calbiochem

at pH 5.2 and 30°C. Sucrose phosphorylase activity was determined essentially as described by Dols et al. *(18)*. Protein was determined as described previously *(17)*.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses were performed in either the Bio-Rad mini-Protean II slab gel system (Bio-Rad, Hercules, CA), using 0.75- or 1.5-mm thick gels *(16,19)*, or the NuPAGE Bis-Tris gel system (Novex, San Diego, CA), with the MOPS-SDS running buffer. GTF activity was visualized *in situ* as described previously *(16,19,20)*. Briefly, gels were washed in buffer (50 m*M* sodium acetate, pH 5.2; 2 m*M* $CaCl_2$; 0.02% sodium azide; 1% Tween-80) to remove SDS, incubated overnight in the same buffer containing 50 mg of sucrose/mL, and stained for carbohydrate.

Fractionation and Solubilization of Enzyme Activity

Cells were collected by centrifuging cultures for 30 min at 10,000*g* and 4°C, resuspended in either buffer A (50 m*M* sodium acetate, pH 5.2; 2 m*M* $CaCl_2$; 0.02% sodium azide) or buffer B (50 m*M* MES, pH 5.5; 150 m*M* NaCl; 2 m*M* $CaCl_2$; 0.02% sodium azide), and then frozen. Suspensions were thawed and diluted for use. Aliquots were mixed with test compounds in microcentrifuge tubes at a final concentration of 50 m*M* MES-NaOH, pH 6.0; 200 m*M* NaCl; and additives at the indicated concentrations. Tubes were kept at room temperature for 10–15 min, and then chilled on ice and stored at 4°C for 3 to 4 d or held at 30°C for 2 h, with occasional mixing by inversion. Half of each suspension was transferred to a clean tube and centrifuged (15,000*g*, 3 min). Suspensions and supernatants were assayed for GTF activity as already described. If necessary, supernatant fractions were concentrated by ultrafiltration using membranes having nominal cutoffs of 100 kDa.

For cell fractionation, a 1-L culture of strain B-1355 was grown in glucose medium in a Fernbach flask and harvested as already described. The cell pellet (6.7 g wet wt) was washed twice in 50 m*M* MES, pH 5.5; 150 m*M* NaCl; 2 m*M* $CaCl_2$; and 0.02% sodium azide (buffer B). It was then resuspended in 3.5 mL of 50 m*M* sodium acetate buffer (pH 5.2), containing 2 m*M* $CaCl_2$ and 0.02% sodium azide (buffer A), and passed four times through a French press at 1.38×10^5 kPa to lyse the cells. The crude lysate was centrifuged for 20 min at 20,000g_{max}. The pellet (P-20) was washed by resuspension in the same buffer. The supernatant fraction from the first centrifugation (S-20) was spun in a TLA 100.3 rotor of a Beckman TL-100 ultracentrifuge (Beckman Instruments, Fullerton, CA) at 109,000g_{max} (*k* factor = 49) at 4°C for 1 h. The resulting clear yellow supernatant fraction (S-100) was collected, and the pellet (P-100) was resuspended in buffer A.

Results

Strain LC-17 produced high levels of GTFs on media containing 2% (w/v) glucose, precluding formation of dextran, which complicated inter-

pretation of earlier results *(6–8)* with strains cultured in sucrose medium. Since much of the GTF activity in our glucose-grown cultures was found in the pellet fraction and dextran is not synthesized without sucrose present, we concluded that the enzyme was associated with the cells themselves.

GTF Distribution in Cell Fractions

To examine the distribution of individual types of GTFs within cell fractions, it was desirable to use a strain that produces alternansucrase. We showed previously that alternansucrase is associated with the cell pellets in sucrose cultures *(17)*, where dextrans were formed. Our recent results showed *(15)* that alternansucrase behaves similarly in cells under conditions in which dextrans are not formed. However, the distribution of GTFs in subcellular fractions, particularly whether association of alternansucrase with cells represents soluble protein sequestered in the cytoplasm or protein attached to the cell wall or cell membrane, is not known. Because strain LC-17 produces little or no alternansucrase *(17)*, we used strain B-1355 for this phase of the study. More than 80% of the GTF activity of B-1355 cultures grown in glucose medium sedimented with the cell pellet (Table 2). A marked increase in the total GTF activity of lysate (obtained by disrupting the cells in a French press) over intact cells was observed, suggesting that sucrose transport into stationary-phase cells is slow, or that the fructose produced was metabolized by the intact cells and not detected in our DNS assay. Because sucrose phosphorylase, which produces reducing sugar (fructose) detectable in our DNS assay, catalyzes the initial step in the major alternative pathway of sucrose catabolism in *L. mesenteroides (18)*, we measured sucrose phosphorylase activity in our fractions. Sucrose phosphorylase activity was too low to account for the apparent increase in the rate of liberation of reducing sugar.

Analysis of GTF activity profiles of the fractions by SDS-PAGE (Fig. 1) showed that alternansucrase (GTF-2) was absent from the culture supernatant fraction (lane 2) and became concentrated in the cell pellet (lane 3). Comparison of pellets and supernatant fractions from the lysed cells showed that alternansucrase activity was associated with the pellet fractions P-20 (lane 5) and P-100 (lane 7), but markedly depleted in the corresponding supernatant fractions, S-20 (lane 6) and S-100 (lane 8). The intensity of band(s) of GTF-3 (dextransucrase) showed fewer differences among fractions than did bands of alternansucrase activity. The concentration of alternansucrase in the particulate fractions suggests that it is associated primarily with the cell wall or membrane, but does not show whether it is a peripheral (surface) or integral (embedded) constituent.

Solubilization of Cell-Associated GTF

Solubilization of the cell-associated GTF activity in small volumes (≤5% of the original culture volume) under mild conditions can improve yields of enzyme activity and simplify recovery, particularly when cell disruption is not needed. We wished to determine whether GTFs could be

Table 2
Location of GTF Activities in Fractions of Glucose B-1355

Fraction	Volume (mL)	GTF activity[a] (U/mL)	GTF activity[a] (total units)	Protein[b] (mg/mL)	Protein[b] (total mg)	Specific activity (U/mg)	Sucrose phosphorylase (U/mL)[a]
Culture	1060	0.062	66	ND			ND
Supernatant	1050	0.007	7.4	ND			ND
Cell pellet	9	6.9	62.1	17.0	153	0.41	0.24
Lysate	9	39.2	353	23.3	210	1.7	1.7
Centrifugation (20,000*g*, 20 min)							
Pellet (P-20)	6[c]	2.51	15.6	11.3	61	0.26	0.29
Supernatant (S-20)	6	30.6	184	15.2	91	2.0	1.5
Ultracentrifugation (100,000*g*, 1 h)							
Pellet (P-100)	1.0[d]	1.8	1.8	6.2	6.2	0.29	0.05
Supernatant (S-100)	3.2	28.6	91.5	13.5	43.2	2.1	1.2

[a]See text for assay conditions.
[b]Bovine serum albumin was used as the standard. ND, Not detectable in the standard version of the protein assay used.
[c]Resuspended in 4 mL of buffer B.
[d]Resuspended in 0.7 mL of buffer B.

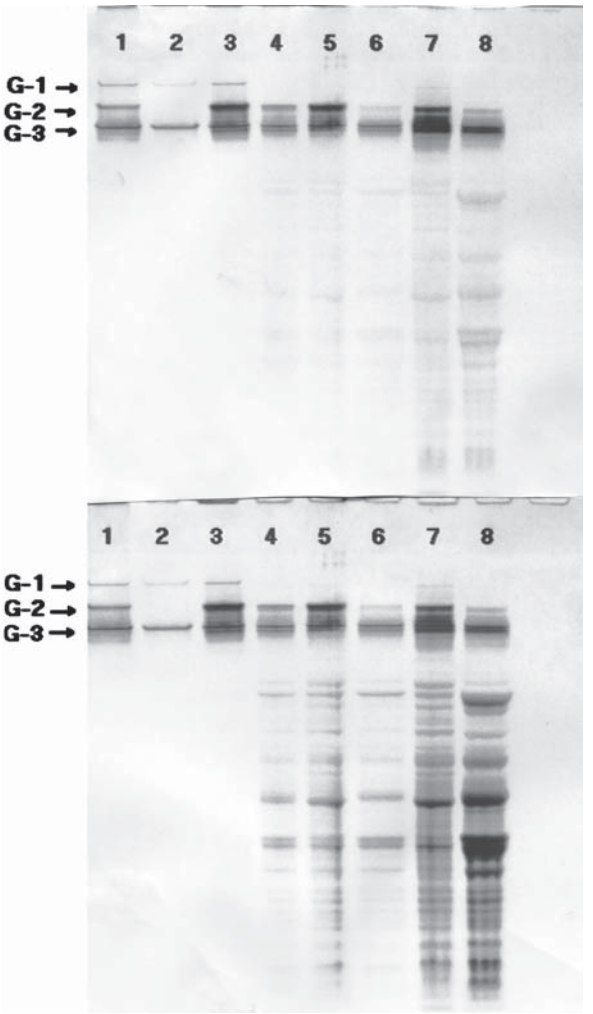

Fig. 1. SDS-PAGE of GTF activity in culture and cell fractions from strain B-1355 grown on GTF medium containing 2% (w/v) glucose. Lane 1, culture; lane 2, culture supernatant; lane 3, cell pellet; lane 4, lysate (broken cells); lane 5, P-20 (20,000*g* pellet from lysate); lane 6, S-20 (20,000*g* supernatant from lysate); lane 7, P-100 (100,000*g* pellet from S-20); lane 8, S-100 (100,000*g* supernatant from S-20). Sample loads contained ≥1 mU of GTF activity (based on DNS assays), except for lanes 1 and 2. Proteins were separated on 4–12% gradient gels, using the Bis-Tris gel system (Novex) as described in the text. GTF activity was detected as described previously *(16,19)*. Principal GTF bands (GTF-1 to GTF-3) are indicated by arrows. **(Top)** GTF activity bands after incubation with sucrose and staining carbohydrate polymers by the periodic acid-Schiff method. Faint staining of rapidly migrating bands is an artifact *(20)* resulting from the heavy protein loads used in these lanes, as revealed by protein staining of the same gel. **(Bottom)** The same gel after subsequent staining for protein with Coomassie blue R250 *(19)*. Because the enzyme detection method is highly sensitive, sample loads needed to provide appropriate levels of GTF activity do not contain detectable amounts of less abundant proteins, and some proteins may be lost during incubation to detect GTF activity. Accordingly, this figure (bottom) reflects the comparative protein heterogeneity of the fractions, not the complete profile.

released from cells under comparatively mild conditions without substantial cell disruption. Lysis by mechanical means or more aggressive chemicals would result in release of intracellular proteins, which would contaminate preparations and might inactivate the GTFs irreversibly, by proteolytic degradation.

A group of dissociating agents was selected, based partly on the results of dissociation or solubilization experiments with other mutants of strain B-1355, for testing on cells of strain LC-17. The main criteria for selection were efficacy and lack of reactivity; hence, potential substrates (such as sucrose fatty acid esters) were excluded. Nondetergent sulfobetaine compounds, introduced recently as solubilizing and stabilizing agents for proteins *(21,22)*, were included. Chaotropes (NaI, NaSCN, GdnHCl, and urea) were also tested.

Chaotropic Agents

The unfolding of GTFs can be reversed under appropriate conditions, since fully active GTF from *Streptococcus mutans (23)* has been obtained following solubilization by 8 *M* urea and subsequent removal, and GTF activities are routinely detected after SDS-PAGE once the SDS has been removed by washing with buffer containing nonionic detergent. In the present experiments, no activity was recovered in the SDS-treated LC-17 cells after addition of Triton X-100 and dilution. This result was puzzling, since Triton X-100 has been used to remove SDS from gels before detection of *S. mutans* GTF *(24)*, and it did not diminish total GTF activity of LC-17 cells (Table 3). However, Triton X-100 concentrations above 50 µg/mL increased the rate of inactivation of dextransucrase of *L. mesenteroides* strain B-512F *(25)*. Attempts to unfold reversibly the GTFs by treatment of LC-17 cell suspensions with 1.5 or 3 *M* GdnHCl, 1 *M* NaI, 1 *M* NaSCN, and 4 or 6 *M* urea, followed by dilution of the denaturant, yielded no GTF activity.

Selected Detergents and Nondetergent Sulfobetaines

Cells from a glucose-grown culture of strain LC-17 (stored in buffer B) were collected by centrifugation, washed once by resuspension in buffer A, and then diluted in buffer B and treated for 2 h at 30°C with a variety of agents (Table 3). Soluble GTF activity of the control suspension represents activity liberated by washing the cells. Other soluble activity values shown are amounts liberated in the first treatment cycle. Solubilization of activity by 1% (w/v) CHAPS, 1% (w/v) SB 3-14, and 1 *M* NDSB201 yielded soluble GTFs having specific activities approximately two- to threefold the control value, suggesting some selectivity in solubilization of GTF relative to total protein. Other agents, notably Triton X-100, SDS, and $iC_{13}E_8$, solubilized more protein but less GTF activity. Total GTF activity in the presence of 25 m*M* NDSBs increased slightly with NDSB195 or NDSB201, but no activity was detected with NDSB256.

The role of the structure of the hydrophobic and hydrophilic moieties of zwitterionic detergents on solubilization of GTFs was tested by varying the structure of one while holding the other constant (Table 4). The carboxy-

Table 3
Solubilization of GTFs from LC-17 Cells[a]

Treatment (30°C, 2 h)			GTF activity[c]			Supernatant protein (μg/mL)[d]	Specific activity of supernatant GTF (U/mg Pr)[e]
			Total	Supernatant			
Addition	Concentration	Concentration/CMC[b]	(U/mL)	(U/mL)	(% of total)		
None	—	—	7.93	0.41	5.2	167[g] (Total: 2120[g]	2.46 Total GTF: 3.74)
Triton X-100	1%	>50	8.48	0.48	5.7	1017	0.47
SDS	1%	~4	0.03[f]	0.44	—	558	0.79
$iC_{13}E_8$	1%	~140	7.21	0.51	7.1	625	0.82
CHAPS	1%	~2	7.81	1.35	17.3	183	7.38
SB 3-10	1%	~1	1.45	0.032	2.2	ND	—
SB 3-12	1%	~10	1.90	0.25	13.4	542	0.47
SB 3-14	1%	>70	4.04	3.17	78.5	600	5.28
Octyl-Glc	1%	~1.4	6.54	0.032	0.5	ND	—
NDSB195	1 *M*	NA	9.08	0.36	4.0	158	2.28
NDSB201	1 *M*	NA	8.98	0.95	10.6	225	4.22
NDSB256	1 *M*	NA	0	0	0	ND	—

[a]Cells were grown in GTF medium containing 2% (w/v) glucose.
[b]NA, not applicable.
[c]Final concentration of added agent present in the assay mixture was 2.5% of that used for treatment, i.e., 0.025% (w/v) or 25 m*M*.
[d]ND, not detectable.
[e]Supernatant fraction. Pr, protein.
[f]Triton X-100 (0.20% [w/w]) was added prior to assay (8:1 ratio to SDS).
[g]Total protein was determined on the untreated suspension, which should be least likely to contain substances that interfere in the dye-binding assay *(17,19)*. Soluble protein was concentrated by precipitation with 0.015% sodium deoxycholate and 10% trichloroacetic acid, as described previously *(19)*. This step also reduces interference by detergent.

Table 4
Solubilization of GTFs by Zwitterionic Detergents Having Different Hydrophilic or Hydrophobic Structures

Detergent (CMC [%])[a]	Concentration (% [w/v])	GTF activity			Soluble protein (mg/mL)	Specific activity of soluble GTF	
		Total (U/mL)	Soluble (U/mL)	Soluble (% of total)		(U/mg)	Relative
None	—	21.8	1.50	6.9	1.37	1.09	1.00
CHAPS (0.5)	0.2	27.7	3.62	13.1	1.48	2.45	2.25
	0.6	25.4	4.65	18.3	1.49	3.11	2.85
	1.0	24.0	4.60	19.2	1.47	3.14	2.88
	2.0	22.3	4.50	20.1	1.61	2.79	2.56
SB 3-14 (0.01)	0.1	22.9	1.93	8.4	2.07	0.93	0.85
	0.5	20.7	6.10	29.5	3.14	1.94	1.78
	1.0	12.9	7.20	55.8	~4.6	~1.5	~1.4
SB 3-12 (0.1)	0.1	24.2	1.29	5.3	1.39	0.93	0.85
	0.5	13.1	1.06	8.0	2.24	0.47	0.43
	1.0	7.69	0.87	11.4	2.45	0.36	0.33
DDMAB (0.13)	0.1	24.2	1.16	4.8	1.64	0.71	0.65
	0.5	1.28	0.33	—			
	1.0	0.49	0.23	—			
Empigen BB (0.05)	0.1	23.9	1.16	4.9	2.04	0.57	0.52
	0.5	<0.2	—	—			

[a]Structure of hydrophobic domain (R): CHAPS, cholamidopropyl (steroid); SB 3-14, tetradecyl (14-C *n*-alkyl chain); SB 3-12, dodecyl (12-C *n*-alkyl chain); DDMAB, dodecyl; Empigen BB, dodecyl. Structure of hydrophilic domain: CHAPS, $RMe_2N^+CH_2CH_2CH_2SO_3^-$; SB 3-14, (same as CHAPS); SB 3-12, (same as CHAPS); DDMAB, $RMe_2N^+CH_2CH_2CH_2COO^-$; Empigen BB, $RMe_2N^+CH_2COO^-$.

late-based detergents were detrimental to total GTF activity at 0.5% (w/v). Sulfobetaines having larger hydrophobic groups (CHAPS, SB 3-14) were the most effective agents in this experiment. CHAPS was effective from 0.4- to 4-fold its critical micelle concentration (CMC), but SB 3-14 was more effective well above its CMC.

Combinations of selected detergents with NDSB201 were tested, because NDSBs can stabilize or protect some proteins or even facilitate renaturation. Specific activity of the soluble GTF was enhanced when LC-17 cells were incubated with NDSB201 (1 *M*) alone or in combination with 1% CHAPS, but not with 1% SB 3-14. High levels of soluble activity were as follows (U/mL, % of total GTF): CHAPS + NDSB201 (8.8, 35%); SB 3-14 (6.8, 59%); SB 3-14 + NDSB201 (6.0, 57%); NDSB201 (5.9, 21%); Triton X-100 + NDSB201 (5.6, 21%); CHAPS (5.3, 21%). Total GTF activity in 1% SB 3-14 ± NDSB201 was ~40% of that of the control. Strong inhibition of total GTF activity by 1% (w/v) deoxycholate was only partially alleviated by NDSB201. Soluble GTF activity in 1% (w/v) Triton X-100 was low (6% of total) when no NDSB201 was added (see Table 3).

SDS-PAGE of samples from glucose-grown cultures incubated at 4°C revealed no major differences among control and treated samples in the patterns of activity bands between total and soluble GTF, after staining the gels for GTF activity.

Discussion

The results described herein and in ref. *15*, obtained with cultures grown on nonsucrose media, provide the first direct demonstration, to our knowledge, of cellular locations of alternansucrase and other cell-associated GTFs of *L. mesenteroides*. The enrichment of alternansucrase in the particulate fractions from lysed cells of strain B-1355 shows, for the first time, that this GTF is associated with either cell walls or membranes. Alternansucrase and dextransucrase differed in distribution between supernatant and particulate fractions. Aggregation of the alternansucrase seems unlikely, because dextransucrase, a similar GTF, did not sediment with the cells in the same way as dextransucrase. Consequently, cell lysis and differential centrifugation may provide an initial fractionation of the GTFs and concentration of alternansucrase in the pellet fraction.

We also found that some GTF activity in cell suspensions can be readily solubilized under mild conditions by extraction with nondenaturing or reversibly denaturing agents, especially CHAPS, SB 3-14, and NDSB201; however, we have not yet optimized conditions for solubilization by a given agent to increase the yield. Of the agents tested, these were the most promising candidates for further testing. In cases in which cost is a factor, the ability to recover and reuse solubilizing agents by dialysis or ultrafiltration would appear to favor CHAPS, owing to its high CMC, or NDSB201, which does not form micelles. In previous work on solubilization of GTFs from cells, maltose was used in the culture medium to favor formation of smaller dextrans and acceptor products *(26)*, because

it was assumed that association of GTFs with cells results from binding to sedimentable dextrans and that these GTFs are basically supernatant enzymes. That approach will solubilize GTF-1 or GTF-3 (dextransucrases) but not alternansucrase (GTF-2).

We did not attempt to determine precisely whether cell-associated GTFs were bound to the cell wall or membrane fraction, but the results are consistent with binding to the cell membrane or an intracellular location. Sequences of GTFs reported so far *(29)* show that they are preponderantly hydrophilic proteins, which seems inconsistent with their being embedded in membranes. The sequence of alternansucrase has not been reported, but substantial homology with other GTFs is expected. Treatment with detergents may have enabled intracellular GTF to pass through leaky cell membranes. If so, other cell constituents should also escape. DNA was largely retained in the cells, because the viscosity of the cell suspensions did not increase appreciably.

GTFs could also be attached by one of two modes of anchorage of cell-surface proteins that have been described in Gram-positive bacteria (*see* ref. *27* and references therein): association of the amino-terminal region with the cytoplasmic membrane, or covalent linkage to cell-wall peptidoglycan at the carboxy-terminus (unlikely for GTFs) *(28)*. Preliminary experiments employing cell-wall lytic enzymes to define more clearly the mechanism of GTF association with the cells suggest that association of GTFs with cells does not involve such attachment. In cultures producing dextran on sucrose medium, it is also possible that dextran could mediate GTF adhesion to the cell surface *(21)* or that GTF could bind to insoluble dextrans. However, our findings (*[15]*; this study) show that glucan binding is not required for cell association of GTFs.

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References

1. Robyt, J. F. (1995), *Adv. Carbohydr. Chem. Biochem.* **51,** 133–168.
2. Jeanes, A. (1986), *Mol. Immunol.* **23,** 999–1028.
3. Jeanes, A., Haynes, S. C., Wilham, C. A., Rankin, J. C., Melvin, E. H., Austin, M. J., Cluskey, J. E., Fisher, B. E., Tsuchiya, H. M., and Rist, C. E. (1954), *J. Am. Chem. Soc.* **76,** 5041–5052.
4. Cote, G. L. (1983), PhD thesis, Iowa State University, Ames.
5. Cote, G. L. and Robyt, J. F. (1982), *Carbohydr. Res.* **101,** 57–74.
6. Dols, M., Remaud-Simeon, M., Willemot, R.-M., Vignon, M., and Monsan, P. F. (1997), *Appl. Biochem. Biotechnol.* **62,** 47–59.
7. Dols, M., Remaud-Simeon, M., Willemot, R.-M., Vignon, M., and Monsan, P. (1998), *Appl. Environ. Microbiol.* **64,** 1298–1302.
8. Castillo, E., Iturbe, F., Lopez-Munguia, A., Pelenc, V., Paul, F., and Monsan, P. (1992), *Ann. NY Acad. Sci.* **672,** 425–430.
9. Paul, F., Lopez-Munguia, A., Remaud, M., Pelenc, V., and Monsan, P. (1992), US Patent 5,141,858.

10. Monsan, P., Paul, F., and Auriol, D. (1995), *Ann. NY Acad. Sci.* **750,** 357–363.
11. Cote, G. L., Leathers, T. D., Ahlgren, J. A., Wyckoff, H. A., Hayman, G. T., and Biely, P. (1997) in *Chemistry of Novel Foods*, American Chemical Society Symposium Series, Okai, H., Mills, O., Spanier, A. M., and Tamura, M., eds., American Chemical Society, Washington, DC, pp. 95–109.
12. Remaud-Simeon, M., Lopez-Munguia, A., Pelenc, V., Paul, F., and Monsan, P. (1994), *Appl. Biochem. Biotechnol.* **44,** 101–117.
13. Smith, E. E. (1970), *FEBS Lett.* **12,** 33–37.
14. Kobayashi, M. and Matsuda, K. (1974), *Biochim. Biophys. Acta* **370,** 441–449.
15. Smith, M. R. and Zahnley, J. C. (1999), *J. Ind. Microbiol. Biotechnol.* **22,** 139–146.
16. Smith, M. R., Zahnley, J., and Goodman, N. (1994), *Appl. Environ. Microbiol.* **60,** 2723–2731.
17. Smith, M. R. and Zahnley, J. C. (1997), *Appl. Environ. Microbiol.* **63,** 581–586.
18. Dols, M., Chraibi, W., Remaud-Simeon, M., Lindley, N. D., and Monsan, P. F. (1997), *Appl. Environ. Microbiol.* **63,** 2159–2165.
19. Zahnley, J. C. and Smith, M. R. (1995), *Appl. Environ. Microbiol.* **61,** 1120–1123.
20. Miller, A. W. and Robyt, J. F. (1986), *Anal. Biochem.* **156,** 357–363.
21. Goldberg, M. E., Expert-Bezançon, N., Vuillard, L., and Rabilloud, T. (1996), *Folding Design* **1,** 21–27.
22. Vuillard, L., Braun-Breton, C., and Rabilloud, T. (1995), *Biochem. J.* **305,** 337–343.
23. Hamada, S., Horikoshi, T., Minami, T., Okahashi, N., and Koga, T. (1989), *J. Gen. Microbiol.* **135,** 335–344.
24. Russell, R. R. B. (1979), *Microbios* **23,** 135–146.
25. Miller, A. W. and Robyt, J. F. (1984), *Biochim. Biophys. Acta* **785,** 89–96.
26. Lopez-Munguia, A., Pelenc, V., Remaud, M., Biton, J., Michel, J. M., Lang, C., Paul, F., and Monsan, P. (1993), *Enzyme Microb. Technol.* **15,** 77–85.
27. Jenkinson, H. F. (1995), *Trends Microbiol.* **3,** 333–335.
28. Schneewind, O., Fowler, A., and Faull, K. F. (1995), *Science* **268,** 103–106.
29. Monchois, V., Willemot, R.-M., and Monsan, P. (1999), *FEMS Microbiol. Rev.* **23,** 131–151.