Biochemical and genetic analysis of Streptococcus mutans \(\alpha\)-galactosidase

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The \textit{aga} gene coding for \(\alpha\)-galactosidase in \textit{Streptococcus mutans} was detected in a recombinant gene library constructed in phage \(\lambda\). The gene was subcloned into plasmid vectors and shown to specify a novel protein of \(M_\text{r} 80000\). Characterization of \(\alpha\)-galactosidase from \textit{S. mutans} and from recombinant \textit{Escherichia coli} expressing \textit{aga} indicated that the enzyme functions as a tetramer. The amino acid composition of the \(\alpha\)-galactosidase, deduced from nucleotide sequencing of \textit{aga}, gave a predicted \(M_\text{r} 82022\) and revealed regions of homology to \(\alpha\)-galactosidases encoded by the \textit{E. coli} Raf plasmids and by \textit{Bacillus stearothermophilus}. Inactivation of the \textit{aga} gene in \textit{S. mutans} resulted in loss of all \(\alpha\)-galactosidase activity and abolished the ability to ferment melibiose; \(\alpha\)-glucosidase activity was also lost, due to an indirect effect on the \textit{dexB} gene.

Introduction

The oral bacterium \textit{Streptococcus mutans} is believed to be the major causative agent of dental caries in humans, a disease in which the tooth enamel is attacked by acid end-products of bacterial metabolism of fermentable carbohydrates (Hamada \& Slade, 1980; Loesche, 1986). Much attention has been focused upon the utilization of sucrose, which can be utilized to form extracellular polymers important in dental plaque formation as well as being used as a fermentable carbon source. However, \textit{S. mutans} is capable of producing acid from a wide range of carbohydrates, which may be derived from the diet or from degradation of host glycoproteins, so the pathways by which these are metabolized are also of interest in explaining the survival and multiplication of \textit{S. mutans} in the oral cavity and the processes leading to dental caries.

We have previously described the cloning and sequencing of the closely-linked \textit{S. mutans} genes \textit{gtaA} and \textit{dexB}. These encode the intracellular enzymes sucrose phosphorylase and dextran glucosidase respectively (Ferretti \textit{et al.}, 1988; Russell \& Ferretti, 1990; Russell \textit{et al.}, 1988). We have now identified a further gene associated with carbohydrate metabolism in the same region of the chromosome which encodes \(\alpha\)-galactosidase (\(\alpha\)-D-galactoside galactohydrolase, EC 3.2.1.22), the gene being named \textit{aga}.

Methods

\textit{Bacteria, vectors and growth media.} \textit{S. mutans} strains were grown in Brain Heart Infusion broth (Oxoid) or the medium described by Terlecki \textit{et al.} (1975) with 0.5\% (w/v) casein hydrolysate instead of the individual amino acids. Carbohydrates (0.5\% w/v) or erythromycin (10 \(\mu\)g ml\(^{-1}\)) were added as required. Conditions for growth and handling of \textit{Escherichia coli} strain JM109, plasmids pUC18 and pUC19 and the single-stranded bacteriophage vectors M13mp18 and M13mp19 were as described by Yanisch-Perron \textit{et al.} (1985). \textit{E. coli} strain M2508 (\textit{melA meta lac}) was kindly provided by Dr R. Schmitt (Schmitt, 1968). Plasmid pV8A91 was kindly provided by Dr F. Macrina (Macrina \textit{et al.}, 1983). \textit{E. coli} strains were grown with shaking in Luria Broth (Maniatis \textit{et al.}, 1982) with antibiotics required as: ampicillin (100 \(\mu\)g ml\(^{-1}\)), erythromycin (400 \(\mu\)g ml\(^{-1}\)).

\textit{Reagents.} Isopropyl \(\beta\)-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl \(\beta\)-D-galactopyranoside (X-\(\beta\)-Gal) were obtained from Northumbria Biologicals. 5-Bromo-4-chloro-3-indolyl \(\alpha\)-D-galactopyranoside (X-\(\alpha\)-Gal) and calfintestinal alkaline phosphatase were from Boehringer Mannheim. T4 DNA ligase was obtained from Pharmacia. Restriction endonucleases were purchased from Northumbria Biologicals and Bethesda Research Laboratories and used according to the manufacturers' instructions. All other reagents and
chemicals were of the highest purity available, and were purchased from Sigma or BDH Chemicals. Initial cloning of aga. As described previously (Russell et al., 1985) a gene library of random fragments of S. mutans strain Ingbrit constructed in bacteriophage λL47.1, was screened on minimal medium containing sucrose or raffinose as carbon source for the presence of recombinant plaques which released monosaccharides and so cross-fed the surrounding E. coli.

Nucleotide sequencing. From λL42, a recombinant of bacteriophage λL47.1 previously shown to carry an insert of chromosomal DNA from S. mutans strain Ingbrit including the gfa gene (Russell et al., 1985) a series of EcoRI fragments were isolated. In the work leading to this report, the 4.48 kb fragment was unidirectionally digested with Bal31 and a series of nested deletion derivatives subcloned into bacteriophage M13 vectors mp18 and mp19 for sequencing by the dideoxy chain-termination method as previously described (Ferretti et al., 1986). All sequences were confirmed from at least two overlapping clones, and the entire sequence was determined on both strands. The sequence information was analysed by the James M. Pustell DNA-Protein sequencing program (International Biotechnologies) and Staden-Plus programs (Amerham). Comparison with protein sequences held in the Protein Information Resource of the National Biological Research Foundation database was by the FASTP program of Wilbur & Lipman (1982).

Subcloning of the aga gene. Inspection of the distribution of restriction sites and coding regions within the nucleotide sequence indicated that the entire open reading frame expected for carrying aga was contained in a 2.7 kb AatII fragment. Such a fragment was therefore isolated from the original recombinant phage λL42 by preparative agarose electrophoresis, the 'sticky ends' filled by use of Klenow fragment of DNA polymerase and the fragment ligated into the Smal sites of plasmid vectors pUC18 and pUC19 to generate recombinant plasmids pSF110 and pSF111. Plasmid pSF106 was constructed by ligating the 4.48 kb EcoRI fragment carrying aga and flanking regions into pUC18.

Agarose gel electrophoresis, electro-elution, dephosphorylation, ligation of DNA and transformation of E. coli cells were as described by Maniatis et al. (1982). Plasmid DNA was routinely prepared using Qiagen Tips (Hybaid, London, UK).

Gene inactivation. Plasmid pSF110 was digested to completion with the restriction enzyme HindIII and fractionated by electrophoresis through a 1.2% (w/v) agarose gel in TBE buffer (90 mM-Tris, 90 mm-boric acid, 2 mM-EDTA) A 0.45 kb internal fragment of the aga gene was excised from the gel, electro-eluted, and purified by sequential extractions with phenol and chloroform. The fragment was ligated with T4 ligase to the plasmid pVA891 (Macrina et al., 1983) which had been linearized with HindIII and dephosphorylated with alkaline phosphatase, to form plasmid pSF122. Plasmid pSF122 was used to transform S. mutans LT1 to erythromycin resistance, essentially as described by Perry & Kuramitsu (1981). S. mutans LT1 is a highly-transformable strain to be described in detail elsewhere (L. Tao, unpublished). The recombinant strain of S. mutans in which aga had been inactivated was named strain 516.

To confirm that plasmid pSF122 had been inserted into aga, chromosomal DNA from S. mutans strains Ingbrit, LT1 and 516 was isolated and digested to completion with the restriction enzyme EcoRI. After agarose gel electrophoresis, the DNA was transferred to Hybond N* membrane as described below and probed with 32P-labelled 4.48 kb EcoRI-insert from plasmid pSF106.

Hybridization of DNA. Chromosomal DNA (2–5 μg) was digested completely with the restriction endonuclease EcoRI, electrophoresed through a 0.8% (w/v) agarose gel, and after pretreatment (Maniatis et al., 1982) the DNA fragments were transferred onto Hybond N* nylon filters (Amersham) by means of a vacuum blotting apparatus (Hybaid) and pre-hybridized in 5 × SSC (Maniatis et al., 1982) containing 1% (w/v) blocking reagent (Boehringer Mannheim), 0.1% (w/v) sodium laurylsarcosine and 0.02% (w/v) SDS at 65 °C. The immobilized DNA was probed with the 4.48 kb EcoRI restriction fragment of plasmid pSF106, which had been labelled with 32P by the random-primer method (DuPont Chemicals). Filters were washed with 2 × SSC plus 0.2% (w/v) SDS for 2 × 15 min and then with 0.2 × SSC plus 0.2% (w/v) SDS for 2 × 15 min. Autoradiograms were prepared using Fuji or Kodak X-OMAT-AR films at −70 °C for 2–16 h.

Fermentation tests. The ability of wild-type and mutant strains of S. mutans to ferment carbohydrates was tested by inoculating wells of a microtiter tray containing Purple Broth Base (16 g L⁻¹, Difco) and Thioglycollate medium (24 g L⁻¹, Difco) with appropriate sugars at 1% (w/v) and incubating anaerobically at 37 °C (Shklar & Keene, 1974).

Enzyme assays. S. mutans or E. coli cells were disrupted by sonication and the cell debris removed by centrifugation at 4 °C and 13600 g for 10 min; phage lysates were prepared as described by Russell et al. (1985). Protein concentration was determined with the Pierce protein assay. Enzyme activities were determined either with whole cells or with cell-free extracts in 50 mM-Tris·HCl buffer, pH 8, containing appropriate substrate in the wells of a microtiter tray. With 2-aminopropionyl galactoside (2-AP-Gal, Sigma) the change in absorbance of the reaction mixture was followed at 405 nm using a Titrettek plate reader. With 4-methylumbel- liferyl α-D-galactoside as substrate, the fluorescence of released methylumbelliferyl was measured at 460 nm (excitation at 380 nm) using a Perkin Elmer fluorimeter with microplate-reading attachment. When raffinose or melibiose was used as substrate, liberated galactose was quantified by coupling the reaction to galactose dehydrogenase at pH 8. The reaction mixture contained 50 mM-Tris·HCl (pH 8), raffinose or melibiose; NAD, 10 mM; galactose dehydrogenase (Sigma), 0.357 units mL⁻¹; cell-free extract protein, 35 μg. Increase in absorbance at 340 nm with time was followed. The absorption coefficient of NADH was taken to be 6.22 × 10³ mol⁻¹ cm⁻¹.

Gel filtration. Samples were applied to a 30 cm × 1 cm column of Sepharose CL-6B-200 (Pharmacia) equilibrated with 50 mM-Tris·HCl (pH 7.5) containing 100 mM- NaCl. Eluted samples were collected into microtiter plates with a Gilson 203 fraction collector. The void volume was determined with Blue Dextran. Thioglycollin (M, 669000), apoferritin (443000), β-amylose (200000), alcohol dehydrogenase (150000), albumin (69000) and β-galactosidase (460000) from an extract of E. coli M2508 were used as M₉ markers. β-Galactosidase was assayed as described for a-galactosidase but with 2-nitrophenyl β-D-galactopyranoside (Sigma) as substrate. Protein concentration was determined as above.

Polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was as described by Laemmli (1970).

Isoelectric focusing. This was done using ultrathin-layer polyacryl- amide gels (Servalyt Precotes 3-10, Serva Feinbiochemica). Calibration was done with the Broad PI kit of coloured standard proteins supplied by BDH. a-Galactosidase activity was visualized by staining with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (N-a-Gal) in 50 mM-sodium phosphate buffer, pH 6.5 (Tubb & Liljestrom, 1986).

Results

Cloning of aga

Screening of the recombinant gene library of the S. mutans chromosome in phage λ on sucrose-containing
medium resulted in the detection of plaques in which sucrase activity was manifested by the stimulation of growth of *E. coli* surrounding the plaques (Russell et al., 1985). The activity in these plaques was found to be due to the expression of the genes *gtfA* or *scrB*, which were subsequently shown to encode sucrose phosphorylase (Russell et al., 1988) or sucrose-6-phosphate hydrolase (Hayakawa et al., 1986; Lunsford & Macrina, 1986) respectively. Some plaques also accumulated polymer and were found to express the *gtfB* gene for fructosyltransferase (Aduse-Opoku et al., 1989) or a *gtf* gene for glucosyltransferase as previously described for another species of oral *Streptococcus* (Gilpin et al., 1985).

To aid in the classification of these various types of sucrose-degrading recombinants, the plaques were rescreened on medium containing raffinose as carbon source. The *α*-linked trisaccharide raffinose is galactosyl-glucosyl-fructose and thus serves as a substrate for *α*-galactosidase, invertase and fructosyltransferase (the latter two enzymes can utilize either sucrose or raffinose). Of the recombinants expressing *gtfA* and so capable of degrading sucrose, approximately 50% could also attack raffinose (Ferretti et al., 1988). However, none formed polymer, indicating that no fructosyltransferase activity was present; there was no additional action on sucrose beyond that due to the presence of *gtfA*, indicating that invertase was also not present. Lysates were therefore prepared from *E. coli* infected with each of a number of recombinant phages and these were all found to have *α*-galactosidase activity detectable with colorimetric or fluorimetric substrates. In order to investigate the properties of the enzyme expressed in *E. coli* from the cloned *α*-galactosidase (*aga*) gene, it was necessary first to determine the properties of *α*-galactosidase in *S. mutans*.

### Properties of *S. mutans α-galactosidase*

By use of the sensitive fluorogenic substrate methylumbelliferyl *α*-D-galactopyranoside, it was possible to detect *α*-galactosidase activity in whole cells or cell-free extracts of *S. mutans* grown on rich medium or on semi-defined medium in which no galactose-containing carbohydrates were present. Marked increases in activity were, however, observed when melibiose (galactosyl-glucose) or raffinose was present as carbon source (Table 1). These results on the induction of *α*-galactosidase in *S. mutans* are thus in agreement with the report of Barletta & Curtiss (1989). Despite the fact that *α*-galactosidase was induced in melibiose-containing medium, growth on this substrate as sole carbon source was only about half of that on glucose, galactose or raffinose (mean generation times of 4.4-5.5 h, as compared to 1.75-2 h). Since maximum activity was found in cell-free extracts of *S. mutans* grown in defined medium with raffinose as carbon source, these extracts were used as source of enzyme to determine the pH optimum and kinetic properties of *α*-galactosidase (see Table 2). Dialysed extracts retained full activity which was not enhanced by the addition of 10 mM-Mn²⁺, Mg²⁺, Cu²⁺, Fe²⁺ or Zn²⁺ or by 10 mM-EDTA. Activity was completely inhibited by exposure of extracts to 100 μM-p-chloromercuriben-
zoic acid, suggesting that a thiol group was essential for activity.

Subcloning of the aga gene

A partial restriction map of the region of S. mutans DNA insert in recombinant phage λS42 is shown in Fig. 1. Preliminary results indicated that aga was located on the 4.48 kb EcoRI fragment and nucleotide sequencing of this fragment (see below) revealed the existence of a long open reading frame (2160 bp). This open reading frame is bounded by two AvaII sites, which allowed the construction of recombinant plasmids pSF110 and pSF111, in which the 2.7 kb AvaII fragment is inserted in opposite orientations downstream of the lac promoter region of pUC plasmid vectors. α-Galactosidase activity could be detected only in extracts of E. coli strain JM109 carrying pSF110, indicating that transcription of aga proceeded in the direction left to right shown in Fig. 1 and that expression required the presence of an E. coli promoter. No activity was detected in extracts of E. coli carrying pSF111, regardless of whether the culture had been exposed to IPTG or not.

SDS-PAGE of proteins from E. coli JM109 carrying pSF110 and exposed to 1 mM-IPTG for 3 h showed the induction of a polypeptide of M., 80006 (Fig. 2). The isoelectric point of α-galactosidase in extracts of both S. mutans Ingbrit and E. coli JM109 carrying pSF110 was at pH 6.1.

In order to characterize the cloned α-galactosidase further, and remove the possibility of background activity from the host cell, pSF110 was introduced into E. coli strain M2508, which has a mutation in the melA gene coding for α-galactosidase (Schmitt, 1968). The α-galactosidase in extracts from the recombinant had properties indistinguishable from those found for α-galactosidase from S. mutans with regard to pH optimum, pI, and K_m for melibiose, raffinose, and the synthetic substrates.

Properties of the cloned α-galactosidase

E. coli M2508 containing plasmid pSF110 was grown overnight in 200 ml LB medium with 1 mM-IPTG, harvested, washed with 50 mM-Tris/HCl buffer, pH 7.5, and resuspended in 10 ml of the same buffer. Cells were sonicated and the supernatant clarified by centrifugation. Cell-free extracts were used in all assays. K_m values were calculated from double reciprocal plots of substrate concentration against rate of reaction and are given in Table 2.

**Table 2. Comparison of the properties of α-galactosidases**

<table>
<thead>
<tr>
<th>Subunit M_r (chromosome)</th>
<th>E. coli MelA* (plasmid)</th>
<th>E. coli RafA† (plasmid)</th>
<th>B. stearothermophilus‡ (plasmid)</th>
<th>S. mutans</th>
</tr>
</thead>
<tbody>
<tr>
<td>M_12062</td>
<td>81188</td>
<td>~80000</td>
<td>82022</td>
<td></td>
</tr>
<tr>
<td>No. of subunits</td>
<td>2</td>
<td>4</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>Cofactors</td>
<td>NAD, Mn²⁺</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>pH optimum</td>
<td>8.1</td>
<td>7.2</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>K_m melibiose</td>
<td>3 mM</td>
<td>0.14 mM</td>
<td>ND</td>
<td>4-4 mM</td>
</tr>
<tr>
<td>K_m raffinose</td>
<td>10 mM</td>
<td>2-2 mM</td>
<td>ND</td>
<td>9.14 mM</td>
</tr>
</tbody>
</table>

ND, Not determined.

* Burstein & Kepes (1971); Liljestrom & Liljestrom (1987); Nagao et al. (1988).
† Schmid & Schmitt (1976); Aslanidis et al. (1989).
‡ Ganter et al. (1988).
Fig. 3. Nucleotide sequence of the *aga* gene of *S. mutans* Ingbrtt and the deduced amino acid sequence of its product. Amino acid residues and nucleotides are numbered on the right of the sequence.
The pH optimum was determined using the fluorogenic substrate in a series of citrate, Sorenson phosphate and Tris/HCl buffers. The enzyme was active in the range 5–7.5, with a pronounced maximum close to pH 6.5. The \( \alpha \)-galactosidase exhibited higher activity in phosphate than in Tris/HCl buffer, activity at pH 7.5 being 1.8 times greater in phosphate than Tris. In contrast to the \( E. \) coli \( \alpha \)-galactosidase (Burstein & Kepes, 1971), there were no discernible differences in \( \alpha \)-galactosidase activity between extensively dialysed and non-dialysed cell-free enzyme preparations. Furthermore, addition of 10 mM-EDTA had no effect. Metal ions (\( \text{Mg}^{2+}, \text{Mn}^{2+}, \text{Cu}^{2+}, \text{Fe}^{2+}, \text{Zn}^{2+} \)), added to 10 mM, had no effect.

When cell-free extracts of \( S. \) mutans or \( E. \) coli carrying plasmid pSF110 were subjected to gel permeation chromatography, \( \alpha \)-galactosidase activity eluted as a single symmetrical peak, with an apparent \( M_r \) close to 300,000. However, since electrophoresis in the presence of SDS indicated that the product of the \( \alpha \)-galactosidase has an \( M_r \) of 80,000, these results suggest that the active enzyme consists of a tetramer of four subunits which are dissociated by SDS.

Nucleotide sequence of \( \alpha \)-galactosidase

The nucleotide sequence of the \( \alpha \)-galactosidase gene is shown in Fig. 3. Analysis of the \( \alpha \)-galactosidase open reading frame reveals that it encodes a 720 amino acid protein with a predominantly hydrophilic character and a predicted \( M_r \) of 82,022. There is no evidence for the existence of a signal peptide resembling those found in proteins which are known to be secreted (von Heijne & Abrahamsen, 1989). Furthermore, the \( M_r \) of the \( \alpha \)-galactosidase product detected by SDS-PAGE in recombinant \( E. \) coli was 80,000 (Fig. 2), close to that predicted from the sequence data, and there was no difference in isoelectric points of \( \alpha \)-galactosidase produced in \( S. \) mutans and in recombinant \( E. \) coli. It therefore appears that the \( \alpha \)-galactosidase gene product does not undergo post-translational modification and it can be concluded to have an intracellular location.

Inactivation of the \( \alpha \)-galactosidase gene in \( S. \) mutans

In order to inactivate the \( \alpha \)-galactosidase gene, we used a strategy similar to that employed by Perry et al. (1985) and Barletta et al. (1988) to inactivate \( gtfA \). A 0.45 kb HindIII internal fragment from within the \( \alpha \)-galactosidase coding region was ligated to plasmid pVA891 also cut with HindIII. The recombinant plasmid was then introduced into \( S. \) mutans strain LT11 by transformation and erythromycin-resistant colonies were selected. Since pVA891 cannot replicate in \( S. \) mutans, resistant colonies are those in which the entire plasmid has inserted into the chromo-

![Fig. 4. DIAGON plot comparing the primary amino acid sequences of the \( \alpha \)-galactosidase encoded by the \( S. \) mutans \( \alpha \)-galactosidase gene and the \( \alpha \)-galactosidase encoded by the \( E. \) coli rotA gene. The numbers of individual amino acid residues are shown on the axes.](image)

some by homologous recombination between the fragment of \( \alpha \)-galactosidase encoded in the plasmid and the chromosomal gene.

In order to confirm the insertion of the plasmid into the chromosome of mutant 516, chromosomal DNA from \( S. \) mutans strains Ingbritt, LT11 and 516 were digested with \( \text{EcoRI} \), subjected to agarose gel electrophoresis and probed by Southern blotting with a fragment including \( \alpha \)-galactosidase. The ethidium-bromide-stained agarose gel showed no apparent differences in DNA banding patterns between \( S. \) mutans strains Ingbritt, LT11 and 516. However, chromosomal integration of pSF122 was confirmed following hybridization; the 4.48 kb \( \text{EcoRI} \) fragment detected by the probe in strain LT11 (identical to that in Ingbritt) was increased in size by about 0.7 kb in mutant 516. This increase was that predicted from the restriction map of pSF122.

Mutant strain 516 in which the \( \alpha \)-galactosidase gene had been inactivated was tested for the presence of \( \alpha \)-galactosidase and for the ability to ferment glucose, fructose, galactose, raffinose, melibiose, sucrose, lactose, isomaltose or isomaltooltriose. No \( \alpha \)-galactosidase activity could be detected in the mutant, showing that \( \alpha \)-galactosidase had indeed been inactivated. Mutant 516 was unable to produce acid from melibiose, showed reduced acid production from raffinose and failed to ferment isomaltose or isomaltotriose. The fermentation of other sugars was unaffected.

Comparison with other \( \alpha \)-galactosidases

The properties of \( S. \) mutans \( \alpha \)-galactosidase are summarized in Table 2, along with those of \( \alpha \)-galactosidases from \( \text{Bacillus stearothermophilus} \) (Ganter et al., 1988), the enzyme encoded by the \( E. \) coli K12 \( \text{mel}A \) gene (Burstein

![Fig. 4. DIAGON plot comparing the primary amino acid sequences of the \( \alpha \)-galactosidase encoded by the \( S. \) mutans \( \alpha \)-galactosidase gene and the \( \alpha \)-galactosidase encoded by the \( E. \) coli rotA gene. The numbers of individual amino acid residues are shown on the axes.](image)
& Kepes, 1971; Liljestrom & Liljestrom, 1987; Nagao et al., 1988; Schmitt & Rotman, 1966) and that encoded by the Raf plasmids found in many wild isolates of E. coli (Schmid & Schmitt, 1976; Schmitt et al., 1979). The S. mutans α-galactosidase shows few properties in common with the E. coli chromosomally-determined enzyme but does resemble the enzyme from B. steaorotherophilus in size and lack of requirement for cofactors. Comparison of the deduced amino acid sequences of the various α-galactosidases revealed no regions of homology between the S. mutans and E. coli chromosomal enzymes but the central region of the S. mutans enzyme and that encoded by the Raf plasmid of E. coli did show strong homology (Fig. 4).

Discussion

Ability to ferment melibiose is characteristic of most isolates of S. mutans and this activity is dependent upon α-galactosidase. We show here that loss of α-galactosidase following insertional inactivation of the aga gene results in inability to ferment melibiose. Fermentation of raffinose is also reduced following loss of α-galactosidase, the residual activity presumably being due to the fructose moiety of raffinose being polymerized by fructosyltransferase to a fructan which is subsequently cleaved by fructanase to release free fructose. Inactivation of aga also leads to loss of ability to ferment the α-glucosides isomaltose and isomaltotriose. We have reported elsewhere (Russell et al., 1991) that inactivation of the dexB gene also abolishes fermentation of isomaltose and isomaltotriose (but does not affect melibiose utilization). The results obtained with the inactivation of aga can be interpreted as a polar transcriptional effect on the downstream dexB gene and we have evidence that the entire region including aga, gtfA and dexB (Fig. 1) constitutes an operon-like arrangement which also includes a series of genes for a binding-protein-dependent transport system (Russell et al., 1991).

α-Galactosidases are not widely known in bacteria, though they have been reported in Streptococcus bovis (Bailey, 1963) and Streptococcus pneumoniae (Li et al., 1963) as well as in E. coli and B. stearotherophilus. They are, however, of considerable interest to plant biochemists and are attracting interest for their possible use in biotechnological processes. The properties of the S. mutans α-galactosidase resemble closely the α-galactosidases found in B. stearotherophilus and there is a strongly-conserved region of amino acid sequence in the centre of the molecules (R. Mattes, personal communication). This conserved region is also found within the α-galactosidase specified by the Raf plasmids of E. coli (Aslanidis et al., 1989). The Raf plasmids confer the ability to transport and metabolize raffinose, carrying genes for a permease, α-galactosidase and sucrose hydrolase (invertase). Aslanidis et al. (1989) suggested that the Raf operon found on the plasmids had a modular construction, having picked up each of the genes from a different source. It is therefore interesting to note the strong similarity between the Raf-plasmid-determined and the S. mutans enzymes, though there appears to be no similarity between the products of the transport and sucrose genes from the two sources. It seems possible that the Raf plasmid α-galactosidase may have originated in a Gram-positive organism, since it shares properties with S. mutans and B. stearotherophilus enzymes and is quite unlike the chromosomally-determined enzyme in E. coli (Table 2).

The linkage of aga, gtfA and dexB raises the possibility that this region of the S. mutans chromosome may contain additional genes concerned with the metabolism or transport of carbohydrates. Evidence from gene inactivation described here and by Barletta & Curtiss (1989) also shows that the control of the three enzyme-coding genes already identified is in some way coordinate; further nucleotide sequencing of this region should help elucidate the mechanisms operating.

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References


FEMS Microbiology Letters 30, 37-41.


Infection and Immunity 56, 2763-2765.


