Comparative kinetic analysis of glycerol 3-phosphate cytidylyltransferase from Enterococcus faecalis and Listeria monocytogenes

Ashley N. Merici
Illinois State University

Jon A. Friesen
Illinois State University

Follow this and additional works at: https://ir.library.illinoisstate.edu/fpchem

Part of the Medicine and Health Sciences Commons

Recommended Citation
Merici, Ashley N. and Friesen, Jon A., "Comparative kinetic analysis of glycerol 3-phosphate cytidylyltransferase from Enterococcus faecalis and Listeria monocytogenes" (2012). Faculty Publications – Chemistry. 5.
https://ir.library.illinoisstate.edu/fpchem/5

This Article is brought to you for free and open access by the Chemistry at ISU ReD: Research and eData. It has been accepted for inclusion in Faculty Publications – Chemistry by an authorized administrator of ISU ReD: Research and eData. For more information, please contact ISUReD@ilstu.edu.
Comparative kinetic analysis of glycerol 3-phosphate cytidylyltransferase from *Enterococcus faecalis* and *Listeria monocytogenes*

Ashley N. Mericli, Jon A. Friesenii

Department of Chemistry, Illinois State University, Normal, IL, U.S.A.

Source of support: Departmental sources

Summary

Background: Glycerol 3-phosphate cytidylyltransferase (GCT) is an enzyme central to the synthesis of teichoic acids, components of the cell wall in gram positive bacteria. Catalysis by GCT from *Enterococcus faecalis* and *Listeria monocytogenes* has been investigated and catalytic properties compared.

Material/Methods: The genes encoding GCT were cloned from genomic DNA and recombinant proteins expressed in *E. coli* and purified. Enzyme assays were used to determine kinetic constants $k_{\text{cat}}$ and $K_m$. Chemical crosslinking provided a means to assess quaternary structure of each GCT.

Results: Recombinant *Enterococcus faecalis* GCT had an apparent $k_{\text{cat}}$ value of 1.51 s$^{-1}$ and apparent $K_m$ values of 2.42 mM and 4.03 mM with respect to substrates cytidine 5'-triphosphate (CTP) and glycerol phosphate. *Listeria monocytogenes* GCT had an apparent $k_{\text{cat}}$ value of 4.15 s$^{-1}$ and apparent $K_m$ values of 1.52 mM and 6.56 mM with respect to CTP and glycerol phosphate. This resulted in $k_{\text{cat}}/K_m$ values of 0.62 s$^{-1}$mM$^{-1}$ and 0.37 s$^{-1}$mM$^{-1}$ for *E. faecalis* GCT and 2.73 s$^{-1}$mM$^{-1}$ and 0.63 s$^{-1}$mM$^{-1}$ for *L. monocytogenes* GCT with respect to CTP and glycerol phosphate, respectively.

Conclusions: The genome of both *Enterococcus faecalis* and *Listeria monocytogenes* contain a gene that encodes a functional GCT. The genes are 67% identical at the nucleotide level and the encoded proteins exhibit a 63% amino acid identity. The purified, recombinant enzymes each appear to be dimeric and display similar kinetic characteristics. Studying the catalytic characteristics of GCT isoforms from pathogenic bacteria provides information important for the future development of potential antibacterial agents.

key words: catalysis • cytidylyltransferase • kinetics

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=883535

Word count: 3430

Tables: 1

Figures: 8

References: 24

Author's address: Jon A. Friesen, Department of Chemistry, 4160 Chemistry, Illinois State University, Normal, IL 61790-4160, U.S.A., e-mail: jfriese@ilstu.edu
**BACKGROUND**

*Enterococcus faecalis* is a Gram-positive bacterium found in the gastrointestinal tract, oral cavity, and heart lining of humans. This fermentative, facultatively anaerobic bacterium is found in birds, reptiles, insects, plants, water, and soil in addition to mammals [1]. *E. faecalis* is known to cause endocarditis, an inflammation of the heart lining. Vancomycin-resistant *Enterococci* (VRE), first isolated in Europe in 1988, are encountered in the hospital setting and are often treated with drugs such as linezolid, daptomycin, and tigecycline [2,3]. *Listeria monocytogenes*, also a Gram-positive, facultative anaerobic bacterium, is commonly found in foods and ingestion can cause the food borne illness listeriosis, potentially resulting in a blood infection or meningitis and miscarriage in pregnant women [4,5]. The treatment for *Listeria* infections is usually ampicillin or penicillin G combined with an aminoglycoside [6].

Growth of most Gram-positive bacteria is dependent on the synthesis of teichoic acids, major components of the bacterial cell wall. Glycolyl-3-phosphate cytidylyltransferase (*GCT*, *EC 2.7.7.39) catalyzes the transfer of the cytidyl group of cytidine 5'-triphosphate (CTP) to glycerol 3-phosphate (Figure 1) and is part of a larger pathway that results in the synthesis of teichoic acid poly (glycerol phosphate). The genes concerned with the synthesis of teichoic acid poly (glycerol phosphate) in *Bacillus subtilis* are organized into two divergently transcribed operons, tagAB and tagDEF [7]. A gene of the tagDEF operon, tagD, encodes *GCT* [8]. *GCT* is part of a larger family of cytidylyltransferases, enzymes that catalyze reversible reactions in which an alcohol and CTP are the substrates, and pyrophosphate and a cytidyl ester are the products. There are three principal members in the cytidylyltransferase family, CTP: phosphocholine cytidylyltransferase (*CCT*), CTP: phosphoethanolamine cytidylyltransferase (*ECT*), and CTP: GCT [9]. *CCT* is a major regulatory enzyme in the CDP-choline pathway, which results in the synthesis of phosphatidylcholine in eukaryotes. In addition to its catalytic domain, *CCT* contains a phosphorylated carboxy terminus and is regulated by activation following association of a lipid binding domain with the membrane [10]. *ECT* is part of the CDP-ethanolamine pathway in eukaryotes and is involved in the synthesis of phosphatidylethanolamine. Inspection of the primary sequence of *ECT* suggests it is comprised of two catalytic domains [11]. *GCT* is the smallest member of the cytidylyltransferase family and appears to function as a homodimer. The genes encoding *GCT* from *Bacillus subtilis* and *Staphylococcus aureus* have previously been cloned and each protein expressed in *E. coli*. The three-dimensional structure of *GCT* has been solved for both of these recombinant forms of the enzyme using X-ray crystallography and serve as model structures for the catalytic domain of cytidylyltransferases [9,12,13].

The goal of the research presented here was to clone the genes encoding *GCT* from the pathogenic Gram-positive bacteria *Enterococcus faecalis* and *Listeria monocytogenes*, express the recombinant proteins in *E. coli*, and characterize the kinetic properties of each enzyme. Kinetic parameters of the two recombinant *GCT* isoforms were compared to each other and with the previously reported values for other isoforms of this important enzyme.

**MATERIAL AND METHODS**

**Chemicals**

Activated charcoal, sn-Glycerol 3-phosphate bis(cyclohexylammonium) salt, cytidine 5'-triphosphate (CTP) disodium salt, T7 forward primer (5'-TAATACGACTCACTATAGGG-3'), and T7 reverse primer (5'-GCTAGTTATTTGCTAGGGGG-3') were obtained from Sigma Aldrich. Deoxynucleobonucleotide triphosphate (dNTP) mix, Luria-Bertani (LB) broth, isopropyl β-D-1-thiogalactopyranoside (IPTG), ammonium peroxidate (APS), N,N,N,N-Tetramethylethlenediamine (TEMED), acrylamide, imidazole, trichloroacetic acid (TCA), and ScintiSafe™ scintillation fluid were purchased from Fisher Scientific. Pfx DNA polymerase, SYBR® Green DNA gel stain, and DH5α competent cells were purchased from Invitrogen. *Enterococcus faecalis* genomic DNA (ATCC® 700802™) was obtained from the American Type Culture Collection. *Listeria monocytogenes* EGD+ genomic DNA was purified from a bacterial cell culture obtained from Dr. Brian Wilkinson, School of Biological Sciences, Illinois State University, New England Biolabs was the source of BamHI, Xhol, and T4 DNA ligase and BL21 (DE3) RIL competent cells were obtained from Agilent Technologies. The pET45b vector was from Novagen and BigDye® Terminator sequencing kit was obtained from Applied Biosystems. Low Range SDS-PAGE protein standards and BioRad™ Protein Assay Concentrate were purchased from BioRad. TALON® Metal Affinity Resin was obtained from Clontech. Disuccinimidyl glutarate (DSG) and Dimethyl Suberimidate (DSP) were purchased from Pierce. [14C] glycerol 3-phosphate was obtained from Amersham Biosciences.

**Amplification of the genes encoding GCT**

PCR was used to amplify genes encoding *GCT* using *Enterococcus faecalis* or *Listeria monocytogenes* genomic DNA as a template. For amplification of the gene encoding *E. faecalis* GCT the 5' oligonucleotide had the sequence 5'-TACTGGAATCCAAAAATATTTGTTACG-3' and the 3' oligonucleotide was 5'-TACTCTGGAATTATCCATAATAAATTC-3'. Restriction enzyme sites BamHI and Xhol (underlined) were added for cloning into pET45b. For amplification of the gene encoding *L. monocytogenes* GCT the 5' and 3' oligonucleotides were 5'-TGAGCAATGGGATCCAAAAAGTTTATCATATGG-3' and 5'-GGGATTACCCCCTGGGCGTTGACCTATT TAGTTCACTGTATATTTG-3'. Restriction enzyme sites BamHI and NotI (underlined) were added for cloning into pET45b. In addition to approximately 100 ng of template DNA, the following were added to each PCR reaction in a final volume of 100 μl: 0.2 mM of each dNTP, 0.1 μM of each oligonucleotide, and 3 units of Pfx DNA polymerase. MgSO₄ concentration was varied from 0.5 mM to 6 mM. Thermocycler parameters were 30 cycles of 94°C for 15 seconds, 45°C for 30 seconds, and 68°C for 60 seconds. Following PCR the DNA was digested with BamHI and Xhol (*E. faecalis* gct) or BamHI and NotI (*L. monocytogenes* gct) and cloned into pET45b. The nucleotide sequence of each gene was verified by sequencing using an Applied Biosystems 3130 Genetic Analyzer and the BigDye® Terminator sequencing kit. The resulting pET45b vectors containing either the gene encoding *E. faecalis* or *L. monocytogenes* GCT were termed pET45b-EF-GCT and pET45b-LmGCT. Cloning appended an additional
24 amino acids at the amino terminus (MAHHHHHHVGT GSNDKSSPSHDH) that contained a 6x-His-tag.

Expression of GCT in E. coli

The BL21(DE3)RIL strain of E. coli was transformed with recombinant pET45b-EfGCT or pET45b-LmGCT and grown at 37°C in LB media containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol. One liter cultures were grown at 37°C while shaking at 250 rpm until an optical density of 0.8 was reached at 600 nm. Induction of protein production was accomplished by the addition of IPTG to a final concentration of 1 mM. Cells were collected by centrifugation at 5,000 × g for 5 minutes and resuspended in 20 ml lysis buffer (20 mM Tris-Cl, 100 mM NaCl, pH 7.5). Protease inhibitor cocktail (Sigma Aldrich) was added so that the final concentration of protease inhibitors were 20 µM AEBSF, 0.003 µM aprotinin, 1.3 µM bestatin, 10 µM EDTA, 0.14 µM E-64, and 0.01 µM leupeptin. Cells were lysed at 10,000 psi using a French Press, the cell extract was centrifuged (27,000 × g, 20 minutes, 4°C), and the supernatant, which contains recombinant GCT, was collected. A 15% SDS-PAGE gel was used to analyze protein expression. After electrophoresis at 150 mV for 70 minutes the gel was stained with a solution containing 0.1% Coomassie Blue, 10% methanol, and 45% acetic acid for 20 minutes. The gel was destained using a solution of 10% methanol and 45% acetic acid until the regions of the gel not containing protein were sufficiently devoid of stain.

Purification of GCT using affinity chromatography

Purification utilized the incorporated 6x-His-tag and a 3 mL column of TALON® Metal Affinity Resin equilibrated with lysis buffer. The supernatant was passed through the column followed by 50 mL of lysis buffer and a second wash with 50 mL of lysis buffer containing 10 mM imidazole. Recombinant GCT was eluted from the column.
using 10 mL of lysis buffer containing 150 mM imidazole. Ten 1 mL fractions were collected from the column and protein concentration determined using the BioRad™ Protein Assay kit and bovine serum albumin (BSA) as standard. SDS-PAGE was used to assess the purity of the protein samples.

Enzymatic assay of purified GCT

The GCT enzyme assay was conducted in 20 mM Tris-HCl pH 8 that contained 6 mM magnesium chloride and indicated concentrations of the substrates CTP and [14C]glycerol phosphate in a final volume of 100 µL. A control tube containing substrates and buffer, but no enzyme, served as background. Assays were conducted at 37°C for 15 minutes, terminated by the addition of 100 µL of 10% (v/v) TCA, placed on ice, and 0.5 mL of a 10 mg/mL activated charcoal suspension was added. After incubation on ice for 15 minutes, tubes were centrifuged for 3 minutes at 16,000 × g, the supernatant was removed, and the charcoal was washed twice with 600 µL of H2O. After washing, the bound [14C]CDP-glycerol product was eluted from the charcoal by incubation in 0.5 mL of 10% (v/v) acetic acid at 37°C for 5 minutes. Following incubation, the tubes were centrifuged at 16,000 × g for 3 minutes, the supernatant was removed and placed in a scintillation vial along with 5 mL of scintillation fluid, and radioactivity determined in a Beckman LS6500 scintillation counter. For kinetic analysis CTP concentration was varied while glycerol phosphate concentration was held constant at 20 mM and glycerol phosphate concentration was varied while CTP was held constant at 10 mM.

Crosslinking of GCT

Crosslinking reactions were conducted using Disuccinimidyld Glutarate (DSG) and Dimethyl Suberimidate (DMS). Reaction tubes contained 0.25 mM purified GCT and either 0 mM, 0.4 mM, or 2 mM crosslinker in a total volume of 100 µL. Following incubation at 22°C for 30 minutes 15% SDS-PAGE was used to analyze the crosslinking reactions.

RESULTS

Expression and purification of GCT using metal affinity chromatography

The pET45b plasmid encodes a 6-histidine (6x-His) tag that was added to the amino terminus of E. faecalis GCT (EfGCT) and L. monocytogenes GCT (LmGCT), affording the use of metal affinity column chromatography to purify each recombinant GCT. Expression of each GCT was robust in E. coli, with an overexpressed band evident on SDS-PAGE at the expected molecular mass of 16.4 kDa for EfGCT and 17.2 kDa for LmGCT (Figure 2, lanes 2 and 5). Each protein was soluble in the cell supernatant liquid following expression and centrifugation. Purification yielded ample amounts of protein, with over 50 mg of purified protein produced per liter culture of E. coli. To assay purity of the protein eluted from the column, purification fractions were analyzed via SDS-PAGE. The gel indicates each GCT was successfully purified (Figure 2, lanes 4 and 7).

Catalysis by EfGCT and LmGCT

A radioisotope assay monitoring conversion of [14C]glycerol phosphate to [14C]CDP-glycerol was used to assess catalytic activity of EfGCT and LmGCT [8]. During catalysis, glycerol 3-phosphate acts as a nucleophile, attacking the α-phosphorous of the CTP, which leads to a putative pentacoordinate phosphorous transition state (Figure 1). To obtain apparent kcat and Km values for EfGCT and LmGCT the concentrations of glycerol phosphate and CTP were each varied and specific activity determined at each concentration. Data were analyzed using the Michaelis-Menten equation and nonlinear regression analysis (Figures 3, 4). Apparent Vmax and Km values were obtained from the curve fitting. Vmax values were then converted to kcat values for comparison to km values reported for other bacterial GCT isoforms. Analysis revealed an apparent km of 1.51 s-1 for EfGCT and an apparent Km values of 2.42 mM with respect to CTP and 4.03 mM with respect to glycerol phosphate. LmGCT exhibited an apparent km of 4.15 s-1 and apparent Km values of 1.52 mM and 6.56 mM with respect to CTP and glycerol phosphate, respectively (Table 1).

Quaternary structure of GCT isoforms

Cross linking reactions using two different chemical crosslinkers were employed to indicate quaternary structure of EfGCT and LmGCT. Disuccinimidyld Glutarate (DSG) contains a N-hydroxysuccinimide (NHS ester) and creates 7.7 Å (5 atom) cross links between amino groups in proteins. Dimethyl suberimidate (DMS) creates a 11.0 Å (8 atom
long) cross link between amino groups in proteins via an imidoester group at each end of the spacer arm. Analysis of cross-linking reaction using SDS-PAGE indicated the presence of a band at approximately twice the monomeric molecular weight of either EfGCT or LmGCT in the presence, but not the absence, of crosslinkers (Figure 5).

**DISCUSSION**

**E. faecalis** and **L. monocytogenes** GCT gene sequences

The nucleotide sequence of the genomes of both *E. faecalis* (accession AE016830) and *L. monocytogenes* (accession NC_003210) have been determined and sequences that encode a putative GCT have been annotated for each organism. In our study the nucleotide sequence of the genes cloned agreed with the database sequences. Comparison of the 399 bp *E. faecalis* GCT gene with the 384 bp *L. monocytogenes* GCT gene reveals 256 of the 384 nucleotides in the *L. monocytogenes* GCT gene sequence match the corresponding nucleotide in the *E. faecalis* GCT gene sequence for a 67% identity. GCT isoforms from *Bacillus subtilis* (accession AM260209) and *Staphylococcus aureus* (accession NC_009632) have been

| Organism            | $K_m$ (mM) CTP | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (s$^{-1}$mM$^{-1}$) |
|---------------------|---------------|----------------------|----------------------------------|
| *Enterococcus faecalis* | CTP: 2.42 GP: 4.03 | 1.51                 | CTP: 0.62 GP: 0.37 |
| *Listeria monocytogenes* | CTP: 1.52 GP: 6.56 | 4.15                 | CTP: 2.73 GP: 0.63 |
| *Staphylococcus aureus* [22] | CTP: 0.036 GP: 0.021 | 2.6 | CTP: 72 GP: 130 |
| *Bacillus subtilis* [15] | CTP: 1.39 GP: 1.09 | 18.8 | CTP: 13.5 GP: 17.2 |

Table 1. Comparison of kinetic parameters for GCT isoforms.

![Figure 3. Plots of EF-GCT activity as a function of substrate concentration for kinetic analysis. The initial reaction velocity (specific activity) as a function of the substrate concentration CTP (A) or glycerol phosphate (B). Data were fit to the Michaelis Menten equation using nonlinear regression analysis and the apparent $K_m$ and $k_{cat}$ values obtained from the curve fitting. The units of specific activity are nmoles CDP-glycerol formed per minute per mg of enzyme.](image-url)
the most extensively studied to date [8,9,12–16]. The *L. monocytogenes* GCT gene sequence has greater identity to both *S. aureus* and *B. subtilis* than to *E. faecalis* while the *E. faecalis* GCT gene sequence is 66–67% identical to each of the other three GCT genes. In an alignment of all four GCT gene sequences there is an overall identity of 50% at the nucleotide level. A notable difference between the four gene sequences is the insertion of six nucleotides in the *E. faecalis* gene sequence, TTGAA found at positions 311–315 and G at position 328. The five nucleotides from 311–315 shift the reading frame temporarily, with the additional G inserted at position 328 restoring the reading frame relative to the other three GCT gene sequences.

Comparison of *EfGCT* and *LmGCT* catalysis to other GCT isoforms

Both recombinant *EfGCT* and *LmGCT* exhibited significant catalytic activity, indicating each *tagD* gene did, in fact, encode glycerol 3-phosphate cytidylyltransferase. This provides experimental evidence that the genome of both *Enterococcus faecalis* and *Listeria monocytogenes* contain a gene that encodes a functional GCT. Kinetic parameters for *EfGCT* and *LmGCT* were compared to previously characterized GCT isoforms from *B. subtilis* and *S. aureus* (Table 1). Analysis reveals that *EfGCT* and *LmGCT* are quite similar to each other with respect to kinetic parameters, with only a 1.6-fold greater CTP $K_m$ for *EfGCT* and 1.6-fold greater glycerol phosphate $K_m$ for *LmGCT*. When compared to kinetic parameters reported for GCT from *B. subtilis*, *K_m* values for *EfGCT* and *LmGCT* are comparable, however, *S. aureus* GCT has been reported to have *K_m* values 50-fold to 300-fold lower than those determined for *EfGCT* or *LmGCT*. With respect to $k_{cat}$, *B. subtilis* GCT has the highest reported value, approximately 5-fold to 12-fold greater than *S. aureus* GCT, *EfGCT*, or *LmGCT*. Since the apparent $k_{cat}$ and $K_m$ values for *EfGCT* and *LmGCT* are similar, the ratio of $k_{cat}/K_m$, a measure of catalytic efficiency, is similar. *B. subtilis* GCT, due primarily to its greater $K_m$, has a catalytic efficiency 5-fold to 50-fold greater than either *EfGCT* or *LmGCT*. *S. aureus* GCT, due primarily to its low $K_m$ values, exhibits catalytic efficiencies 25-fold to 350-fold greater than either *EfGCT* or *LmGCT*.

*EfGCT* and *LmGCT* are dimeric

Cross linking reactions indicated the presence of a band on SDS-PAGE of approximately 35 kDa in the presence, but not the absence, of crosslinkers (Figure 5). Both *EfGCT* and *LmGCT* are about 15 kDa, with an extra 3 kDa contributed by the N-terminal sequence containing the 6x-His-tag. Therefore, a band of approximately 35 kDa indicates the crosslinking of two monomers. These data indicate both *EfGCT* and *LmGCT* are dimeric. Three-dimensional structures of *B. subtilis* and *S. aureus* GCT indicate these forms of GCT are also dimeric [12,13]. The homodimeric forms of the enzyme contain two active sites per dimer. In addition, binding constants have been determined for *B. subtilis* GCT and indicate negative cooperativity of substrate binding [17]. The data from this previous study indicate that efficient catalysis will only occur when both active sites of a dimeric form of GCT are bound to substrate. Since these data indicate a monomer will be catalytically impaired, we infer that dimerization of *LmGCT* and *EfGCT* is likely necessary for efficient catalysis to occur as well.

Analysis of the *E. faecalis* and *L. monocytogenes* GCT amino acid sequences

Comparison of the 132 residue *E. faecalis* GCT amino acid sequence with the 127 residue *L. monocytogenes* GCT amino acid sequence reveals a 65% identity between the two sequences.
L. monocytogenes GCT has greater identity to both S. aureus and B. subtilis GCT, 69% and 72% identity, respectively, than to E. faecalis GCT. However, E. faecalis GCT, however, has a lower identity with respect to S. aureus and B. subtilis GCT, 59% and 62%, respectively, than to L. monocytogenes GCT (Figure 6). In an alignment of all four GCT amino acid sequences there is an overall identity of 46% (Figure 7).

The molecular mass of the two forms of GCT studied here differs slightly with the 132 amino acid EfGCT having a calculated molecular mass of 15,440 Daltons and the 127 amino acid LmGCT a mass of 15,060 Daltons. EfGCT contains an additional 3 amino acids at the carboxy terminus and two inserted amino acids when aligned with LmGCT, found in the region between amino acids 103 and 109 due to the insertion of nucleotides 311-315 and 328 (Figure 7). There are three regions of sequence that have been found to be highly conserved across nearly all of the known GCT sequences and are also present in LmGCT and EfGCT (Figure 7). The first is the sequence ‘GX(Y/F)DXHGXH’ (where X is any amino acid) that contains catalytically important histidines. Previous studies showed that mutating histidine 14 or histidine 17 to alanine in B. subtilis GCT caused a decrease in V_{max} values of more than three orders of magnitude [14]. A second conserved sequence is ‘RTXGSTT’, a region in the structure of B. subtilis GCT that wraps around the nucleotide portion of CTP to form interactions that contribute to the specificity of the enzyme. These two sequences comprise one side of the active site bowl. The conserved ‘RYVDEVI’ sequence, distant from the CTP-binding site, is found towards the bottom of the B. subtilis GCT monomer and is part of the dimer interface [13].

Two other amino acids shown by site-directed mutagenesis to be important for catalysis in B. subtilis GCT are lysine 44 and lysine 46, also present in LmGCT and EfGCT (Figure 7). In the crystal structure of B. subtilis GCT with bound CDP-glycerol, the positively charged side chains of the lysines interact with the negatively charged phosphates of CDP-glycerol. When these two lysines were experimentally mutated to alanines, there was a threefold increase in the K_{m} values for the substrates CTP and glycerol phosphate and the V_{max} value decreased to approximately 10% of the wild-type value [9].
Modeling the three-dimensional structure of *E. faecalis* and *L. monocytogenes* GCT

The three-dimensional structure of *Bacillus subtilis* GCT has been solved and serves as a model structure for the catalytic domain of cytidylyltransferases [9,13]. To assess the potential similarity of *L. monocytogenes* GCT and *E. faecalis* GCT to *B. subtilis* GCT, SWISS-MODEL (http://swissmodel.expasy.org), an automated comparative protein modeling tool [18–22], was used to model the structure of each protein (Figure 8). The amino acid sequences of LmGCT and EfGCT were submitted and the three-dimensional structure modeled using *Bacillus subtilis* GCT (PDB ID 1N1D) as a template. Models suggest both LmGCT and EfGCT likely adopt a dimeric three-dimensional structure similar to *B. subtilis* GCT. Active site histidines 14 and 17, as well as an active site arginine (113 in LmGCT and 115 in EfGCT), are present at the putative active sites in each enzyme. This suggests the active site architecture and catalytic mechanism are similar to the well characterized *B. subtilis* GCT. In addition, arginine 63, known to be at the interface between the two monomers, is also present at the dimer interface in each modeled structure.

**Conclusions**

Since GCT is an enzyme central to teichoic acid synthesis in Gram-positive bacteria such as *E. faecalis* and *L. monocytogenes*, inhibition of GCT in these potentially pathogenic bacteria could halt infection in humans. GCT expression may be related to pathogenicity, as indicated by a genomic profiling study of *Staphylococcus epidermidis* strains from individuals with ocular infections. Pathogenic *S. epidermidis* isolates from endophthalmitis showed amplification of the genomic region containing tagD, suggesting GCT expression may be a factor for the development of infection [23]. The potential of GCT as a potential drug target is validated by recent study showing a tagD gene deletion in *Bacillus subtilis* grown at physiological temperature. J Bacteriol, 2001; 183: 6688–93

**References:**

1. Roca IN, Siqciera JF Jr, Santos KR: Association of *Enterococcus faecalis* with different forms of perianalicular diseases. J Endod, 2004; 30: 515–20
2. Couvalin P, Vancomycin resistance in Gram-positive cocci. Clin Infect Dis, 2006; 42: S25–34
3. Amyes S: Enterococci and streptococci. Int J Antimicrob Agents, 2007; 29: S43–52
4. Ramaswamy V, Creshence VM, Roytja JS et al: Listeria-review of epidemiology and pathogenesis. J Microbiol Immunol Infect, 2007; 40: 4–13
5. Schlech WF III: Foodborne listeriosis. Clin Infect Dis, 2000; 31: 770–75
6. Charpentier E, Couvalin P: Antibiotic resistance in Listeria spp. Antimicrob. Agents Chemother, 1999; 43: 2103–8
7. Lazarevic V, Abellán FX, Möller SB et al: Comparison of ribitol and glycerol teichoic acid genes in *Bacillus subtilis* W23 and 168: identical function, similar divergent organization, but different regulation. Microbiology, 2002; 148: 815–24
8. Park YS, Sweitzer TD, Dixon JE, Kent C: Expression, purification, and characterization of CTP:glycerol 3-phosphate cytidylyltransferase from *Bacillus subtilis*. J Biol Chem, 1999; 274: 13161–66
9. Park YS, Sweitzer TD, Dixon JE, Kent C: Expression, purification, and characterization of CTP:glycerol 3-phosphate cytidylyltransferase from *Bacillus subtilis*. J Biol Chem, 2001; 41: 37922–28
10. Weber GH, Park SP, Sanker S et al: A prototypical cytidylyltransferase: CPT-glycerol 3-phosphate cytidylyltransferase from *Bacillus subtilis*. Structure 1999;9: 1113-24.
11. Bladergroen BA, Honnejing M, Geelen MJ et al: Cloning and expression of CTP:phosphocholine cytidylyltransferase from Staphylococcus aureus in E. coli. J Biol Chem, 1999; 274: 15416–66
12. Fong DH, Yim VCN, D’Elsa MA et al: Crystal structure of CTP:glycerol 3-phosphate cytidylyltransferase from *Staphylococcus aureus* Examination of structural basis for kinetic mechanism. Biochim Biophys Acta, 2005; 1764: 63–69
13. Weber GH, Park SP, Sanker S et al: A prototypical cytidylyltransferase: CPT: glycerol 3-phosphate cytidylyltransferase from *Bacillus subtilis*. J Biol Chem, 1999; 1348: 75–85
14. Badurina DS, Solli-Juran M, Brown ED: CTP: glycerol 3-phosphate cytidylyltransferase from *Bacillus subtilis*. J Biol Chem, 1997; 272: 15161–66
15. Sanker S, Campbell HA, Kent C, Zuiderswar ERP: Delineation of the allosteric mechanism of a cytidylyltransferase exhibiting negative cooperativity. Nature Struct Biol, 2001; 8: 947–52
16. Badurina DS, Solli-Juran M, Brown ED: CTP: glycerol 3-phosphate cytidylyltransferase (TagD) from *Staphylococcus aureus* catalyzes the cytidyl transfer via an ordered Bi-Bi reaction mechanism with micromolar Km values. Biochim Biophys Acta, 2005; 1646: 196–206
17. Sanker S, Campbell HA, Kent C: Negative cooperativity of substrate binding but not enzyme activity in wild-type and mutant forms of CTP:glycerol 3-phosphate cytidylyltransferase. J Biol Chem, 2001; 41: 37922–28
18. Arnold K, Bordoli L, Kopp J, Schwede T: The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. Bioinformatics, 2006; 22: 195–201
19. Kief F, Arnold K, Kunn E et al: The SWISS-MODEL Repository and associated resources. Nucleic Acids Res, 2009; 37: D387–92
20. Schwede T, Kopp J, Guex N, Peitsch MC: SWISS-MODEL: an automated protein homology-modelling server. Nucleic Acids Res, 2003; 31: 3381–85
21. Peitsch MC: Protein modeling by E-mail. Bio/Technology, 1995; 13: 658-60
22. Duggar K, Chengappa P, Sharm S et al: High-resolution genome profiling differentiated *Staphylococcus epidermidis* isolated from patients with ocular infections and normal individuals. Invest Ophthalmol Vis Sci, 2007; 48: 3295–33
23. Bhavsar AP, Beveridge TJ, Brown ED: Precise deletion of tagD and controlled depletion of its product, glycerol 3-phosphate cytidylyltransferase, leads to irregular morphology and loss of *Bacillus subtilis* grown at physiological temperature. J Bacteriol, 2001; 183: 6688–93

BR434