The serotype c-specific polysaccharide antigen of *Actinobacillus actinomycetemcomitans* NCTC 9710 contains an unusual sugar, 6-deoxy-L-talose, which has been identified as a constituent of cell wall components in some bacteria. Two genes coding for thymidine diphosphate (dTDP)-6-deoxy-L-lyxo-4-hexulose reductases were identified in the gene cluster required for biosynthesis of serotype c-specific polysaccharide. Both dTDP-6-deoxy-L-lyxo-4-hexulose reductases were overproduced and purified from *Escherichia coli* transformed with the plasmids containing these genes. The sugar nucleotides converted by both reductases were purified by reversed-phase high performance liquid chromatography and identified by 1H nuclear magnetic resonance and gas-liquid chromatography. The results indicated that one of two reductases converts dTDP-6-deoxy-L-talose and the other produced dTDP-6-deoxy-L-rhamnose (dTDP-6-deoxy-L-mannose). The amino acid sequence of the dTDP-6-deoxy-L-lyxo-4-hexulose reductase forming dTDP-6-deoxy-L-talose shared only weak homology with that forming dTDP-6-deoxy-L-rhamnose, despite the fact that these two enzymes catalyze the reduction of the same substrate and the products are determined by the stereospecificity of the reductase activity. Neither the gene for dTDP-6-deoxy-L-talose biosynthesis nor its corresponding protein product has been found in other bacteria; this biosynthetic pathway is identified here for the first time.

Capsular polysaccharides are ubiquitous structures found on the cell surfaces of a broad range of bacterial species. The polysaccharides often constitute the outermost layer of the cell, and they have been implicated as virulence factors for many animal and plant pathogens (1). Serotype c-specific antigen of *Actinobacillus actinomycetemcomitans* is 6-deoxy-L-talan, which is acetylated at the O-2 position of 1,3-linked 6-deoxy-L-talose (2). This polysaccharide is considered to play an important role in *A. actinomycetemcomitans* serotype c pathogenesis (3, 4). Polysaccharides consisting of only 6-deoxytalose are rare, although 6-deoxytalose has been reported as a constituent of several microbial polysaccharides (5, 6). Except for serotype c-specific polysaccharide from *A. actinomycetemcomitans*, only the O-chain of lipopolysaccharide from *Rhizobium loti* NZP 2213 has been reported as a homopolymer composed solely of 6-deoxy-L-talose (7). In addition, other serotype-specific polysaccharides of *A. actinomycetemcomitans* also contain rare sugars as constituents of microbial polysaccharides; examples include 1-fucose in serotype b-specific polysaccharide (8) and 6-deoxy-D-talose in serotype a-specific polysaccharide (2).

In 1973, 6-deoxy-L-talose was characterized as an unusual sugar, and the instability of dTDP-6-deoxy-L-talose, which is the activated nucleotide sugar form of 6-deoxy-L-talose, was reported (9). The enzymatic activity of the biosynthetic pathway of this sugar nucleotide in a cell-free extract of *Escherichia coli* O45 was also characterized in that report. Since then, no reports on either the isolation of the enzymes or the identification of the genes involved in the biosynthesis of dTDP-6-deoxy-L-talose have been published.

We recently cloned and characterized gene clusters involved in the synthesis of serotype-specific polysaccharide from *A. actinomycetemcomitans* strain Y4 (serotype b) (10) and strain NCTC 9710 (serotype c) (Fig. 1) (11). dTDP-6-deoxy-L-rhamnose biosynthetic genes in the gene cluster from strain Y4 were identified as *rmlA*, *rmlB*, *rmlC*, and *rmlD*; they code for glucose-1-phosphate thymidyltransferase, dTDP-D-glucose-4,6-dehydratase, dTDP-6-deoxy-D-xylo-4-hexulose-3,5-epimerase, and dTDP-6-deoxy-L-lyxo-4-hexulose reductase, respectively (10, 12). Four genes corresponding to these *rml* genes were also found in the gene cluster from strain NCTC 9710 (11). In comparing these two sets of *rml* genes, the amino acid sequences of the dTDP-6-deoxy-L-lyxo-4-hexulose reductases shared only 58% identity, although the glucose-1-phosphate thymidyltransferases, dTDP-D-glucose-4,6-dehydratases, and dTDP-6-deoxy-D-xylo-4-hexulose-3,5-epimerases exhibited more than 95% identities. We predicted that the *rmlD* homologue coded for dTDP-6-deoxy-L-lyxo-4-hexulose reductase, which catalyzes the production of dTDP-6-deoxy-L-talose, because the stereoselectivity of the reduction determines the direction of synthesis of these two 6-deoxyhexoses. In this study, we report that, contrary to that expectation, one of three serotype c-specific genes (ORF7, ORF8, and ORF9) was identified as the *tll* gene coding for dTDP-6-deoxy-L-lyxo-4-hexulose reductase, which forms dTDP-6-deoxy-L-talose. This gene was subcloned into the vector pMAL, and the gene product was purified and characterized.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Culture Conditions**—*E. coli* DH5α (supE44 ΔlacU169 (de3lacZAM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1) (13) was used for DNA manipulations and as a host strain for pMAL-c derivatives (New England Biolabs). *E. coli* ER2566 (F λ fhuA2 (lon) ompT lacZ:T7 gene1 gal sulA11 ΔmerC-mrr144:IS10 R:merC-73:: miniTn10-TetS2 R:zgb-Tn10(TetS) endA1 (dcm)) was used as a

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**Thymidine Diphosphate-6-deoxy-L-lyxo-4-hexulose Reductase Synthesizing dTDP-6-deoxy-L-talose from *Actinobacillus actinomycetemcomitans***

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host strain for pTYB vectors (New England Biolabs). E. coli BL21 (E. coli B F- dcmompT hsdS B5 mB gal) was used as a host strain for pGEX-6P derivatives (Amersham Pharmacia Biotech). E. coli strains were grown aerobically in 2 × TY broth at 37 °C (13). Ampicillin and chloramphenicol were used at final concentrations of 50 μg/ml and 20 μg/ml, respectively.

DNA Manipulations, PCR, and Sequencing Techniques—DNA fragment preparation, agarose gel electrophoresis, DNA labeling, ligation, and bacterial transformation were performed using the methods described by Sambrook et al. (13). PCR amplification was performed using a GeneAmp PCR System 2400 from Perkin-Elmer. Sequencing was performed using an ABI 373 DNA sequencing apparatus (Perkin-Elmer).

Construction of Plasmids—Oligonucleotide primers for the amplification of rmlA, rmlB, rmlC, and tll from A. actinomycetemcomitans NCTC 9710 chromosomal DNA and rmlD from A. actinomycetemcomitans Y4 chromosomal DNA were designed to introduce appropriate restriction sites for cloning. To construct plasmids for gene expression and protein purification, the following sets of primers were used for amplification: for the rmlD gene, 5′-AACATATAGGATATATTTC-GTC-3′ and 5′-AACCCTGGCGCCAGTCTTCTAATAATTTCT-3′; for the rmlB gene, 5′-AATATTGCTTTATTCTACAGCT-3′; for the rmlA gene, 5′-AACATATGAGGTAAATTTCT-3′; and 5′-AACCCGGGTGTGCATCTCTACAGCT-3′; for the rmlC gene, 5′-AACCATATGAAATATTACAT-3′ and 5′-AACCCTGCGTTTTAAGTTAGCTGACGACT-3′; for the rmlD (NCTC 9710) gene, 5′-ATATCCGGGCGCCTTTAATAATAGGCAGCT-3′ and 5′-ATATCCGGGCGCCTTTAATAATAGGCAGCT-3′; for the tll gene, 5′-CTTCCGGCAGTATTCTGTC-3′; and 5′-AACCCGGGTTTAAAATGTTTTAGCGATCTA-3′. PCR products were cloned into the expression vectors pTYB2 (New England Biolabs) and pMAL-c2 (New England Biolabs). Fusion of the rmlA, rmlB, rmlC, and tll genes to the bacterial expression vector pMAL-c2 was accomplished using the vector pTYB-derivatives, 200-ml cultures of 9710 with the glutathione fusion protein on amylose columns, and its cleavage with Factor Xa were done essentially as described by the manufacturer (New England Biolabs). Production of the RmlD (NCTC 9710)-glutathione S-transferase fusion protein was induced by IPTG and purified according to the manufacturer’s instructions. Glycerol was added to the purified proteins to a final concentration of 50%, and they were stored at −20 °C. The purity of the proteins was checked by SDS-PAGE (4% stacking gels and 12.5% separating gels).

Synthesis of dTDP-6-deoxy-L-talose and dTDP-6-deoxy-L-ribose from dTDP-β-glucose—The reaction mixture, containing 50 mM sodium phosphate buffer (pH 7.2), 12 mM MgCl2, 4 mM α-D-glucose 1-phosphate, 4 mM dTTP, 0.45 unit of inorganic pyrophosphatase (Roche Molecular Biochemicals), and appropriate amounts of the purified rmlA gene product, glucose-1-phosphate thymidylyltransferase, was incubated at 37 °C for 1 h. Conversion of α-D-glucose 1-phosphate to dTDP-β-glucose was detected by reversed-phase HPLC and then the rmlB gene product, dTDP-β-glucose-4,6-dehydratase, was added to the reaction mixture. After analysis of conversion of dTDP-β-glucose to dTDP-6-deoxy-L-xylo-4-hexulose, NADPH (8 mM), the rmlC gene product (dTDP-6-deoxy-L-xylo-4-hexulose-3,5-epimerase), and the rmlD gene product (dTDP-6-deoxy-L-lyxo-4-hexulose reductase), which produces dTDP-6-deoxy-L-ribose, were added to the reaction mixture for the detection of dTDP-6-deoxy-L-ribose synthesis. To detect synthesis of dTDP-6-deoxy-L-talose, NADH (8 mM), the rmlC gene product, and the tll gene product were added to the reaction mixture. Each reaction was analyzed by reversed-phase HPLC.

Detection of Sugar Nucleotides by Reversed-phase HPLC—Conversion of sugar nucleotides was confirmed using reversed-phase HPLC as described by Tonetti et al. (14). Samples (10 μl) diluted 10-fold with distilled water were injected onto a TSKgel ODS-80Ts column (0.46 × 15 cm; Tosoh) with 0.5 mM KH2PO4 as the mobile phase at a flow rate of 1.0 ml/min at 40 °C. The eluate was monitored with a UV detector at 260 nm.

NMR Spectroscopy—Approximately 2 mg of sugar nucleotides were pooled from several HPLC runs on an ODS-80Ts. The sample at each run was immediately cooled on ice, and 4 volumes of cold ethanol was added to the solution to avoid degradation of dTDP-6-deoxy-L-talose in 0.5 M KH2PO4, at room temperature. After removing the excess phosphate by adding ethanol, the solution was concentrated by evaporation and lyophilized. The samples were dissolved in D2O and used for NMR analysis. The NMR analysis was performed within 2 days after purification of the dTDP-hexose, and the sample was stored at −30 °C. 1H NMR spectra were recorded with a Bruker AM400 spectrometer. The measurement was made at 298 K. The chemical shifts were referenced to 3-(trimethylsilyl)propanesulfonic acid at 0.0 ppm. The 1H spectra of 128 scans were recorded with presaturation of HOD resonance at 4.72 ppm. Two-dimensional COSY measurement was performed for signal assignments.

Gas-Liquid Chromatography Analysis—dTDP-6-deoxy-L-talose and dTDP-6-deoxy-L-ribose samples were obtained by the same method as described in Ref. 12. About 2 mg of dTDP-6-deoxy-L-talose or dTDP-6-deoxy-L-ribose was dissolved in 300 μl of 0.1 M HCl. Ampoules containing the solutions were sealed under vacuum and heated at 80 °C for 1 h to hydrolyze the dTDP-sugars; the water and HCl were then evaporated. The pellets were converted into the corresponding tetr-O-acetyl glycoside acetate by the method of Leonetti et al. (15). Each product was characterized by gas-liquid chromatography (model GC-14B; Shimadzu Works) with a fused silica capillary column (CP Sil-88, 0.25 mm × 50 m; Chrompack Inc.) at 200 °C. Approximately 1 μl of the sample was injected, and the split ratio was 1:20. Helium was used as a carrier gas at a flow rate of 0.9 ml/min.
RESULTS

Purification of Enzymes Involved in the Synthesis of dTDP-6-deoxy-l-talose and dTDP-l-rhamnose—To characterize the function of the A. actinomycetemcomitans NCTC 9710 rmlA, rmlB, rmlC, rmlD, and tll and strain Y4 rmlD genes, their protein products were purified to near homogeneity by affinity chromatography (Fig. 2) as described in detail under “Experimental Procedures.” The molecular masses of the denatured polypeptides, determined by SDS-PAGE, of 32.3, 40.0, 20.6, 32.4, 31.9, and 30.7 kDa are in close agreement with the predicted molecular weights of the rmlA, rmlB, rmlC, rmlD (NCTC 9710), rmlD (Y4), and tll gene products, respectively.

Synthesis of dTDP-6-deoxyhexoses from D-Glucose 1-Phosphate and dTTP—Conversion of D-glucose 1-phosphate and dTTP to dTDP-sugars was detected by reversed-phase HPLC (Fig. 3). The elution profile of the reaction mixture containing dTTP, D-glucose 1-phosphate, and the purified gene product of the rmlA, rmlB, rmlC, rmlD (NCTC 9710), rmlD (Y4), and tll gene products, respectively.

The elution profiles were in agreement with those of the mixtures reacted with the rmlA and rmlB gene products (glucose-1-phosphate thymidylyltransferase and dTTP-d-glucose-4,6-dehydratase, respectively) from strain Y4 instead of the corresponding gene products from strain NCTC 9710 (data not shown). Considering these results in combination with high homologies of their amino acid sequences, we decided to use the products of the rmlA and rmlB homologues from strain NCTC 9710 as glucose-1-phosphate thymidylyltransferase and dTTP-d-glucose-4,6-dehydratase, respectively, in further investigations. Addition of either the rmlC, rmlD (Y4), rmlD (NCTC 9710), or tll gene product to the reaction mixture containing D-glucose 1-phosphate, dTTP, and the rmlA and rmlB products did not change its elution profile (data not shown). These results agree with the report that the dTDP-6-deoxy-l-lyxo-4-hexulose intermediate is not released from dTDP-6-deoxy-l-lyxo-4-hexulose-3,5-epimerase in the dTDP-l-rhamnose biosynthetic pathway in E. coli (16). The rmlD (Y4) product converted dTDP-6-deoxy-l-lyxo-4-hexulose to dTDP-l-rhamnose in conjunction with the purified gene product of the rmlC (NCTC 9710) product (Fig. 3D). When the gene product of the rmlD homologue from strain NCTC 9710 was added to the reaction mixture instead of the strain Y4 rmlD product, the elution profile exhibited the same pattern as that of the reaction mixture containing the strain Y4 rmlD product (Fig. 3D). This result also suggests that the rmlA, rmlB, and rmlC genes from strain NCTC 9710 code for glucose-1-phosphate thymidylyltransferase, dTTP-d-glucose-4,6-dehydratase, and dTDP-6-deoxy-l-lyxo-4-hexulose-3,5-epimerase, respectively. The elution profile of a reaction mixture containing D-glucose 1-phosphate, dTTP, NADPH, the rmlABC gene products, and the tll product exhibited only one peak, and its retention time agreed with that of authentic NADPH (data not shown). When NADH instead of NADPH was added to the reaction mixture, two major peaks were observed and the retention time of the second peak (26.5 min) was in agreement with that of authentic NADPH.

![Fig. 2](image-url) Gel electrophoresis of recombinant enzymes purified from E. coli strains transformed with the expression plasmids. About 0.5 μg of each protein was incubated at 100 °C in a water bath for 3 min with 0.1% SDS and 1% 2-mercaptoethanol. Each of the treated solutions was subjected to electrophoresis on a 12.5% SDS-polyacrylamide gel. The gel was stained with Coomassie Blue. Lane 1, the purified gene product of rmlA; lane 2, the purified gene product of rmlB; lane 3, the purified gene product of rmlC; lane 4, the purified gene product of rmlD (NCTC 9710); lane 5, the purified gene product of rmlD (Y4); lane 6, the purified gene product of tll. The positions of molecular mass markers (in kilodaltons) are shown on the left.

![Fig. 3](image-url) Reversed-phase HPLC profiles during synthesis of dTDP-l-rhamnose or dTDP-6-deoxy-l-talose. Samples were injected onto a TSKgel ODS-80Ts column. A, no enzyme was added to the reaction mixture. B, the purified rmlA gene product was added to the reaction mixture. C, the purified rmlA and rmlB gene products were added to the reaction mixture. D, NADPH and the purified rmlA, rmlB, rmlC, and rmlD (NCTC 9710) gene products were added to the reaction mixture. E, NADPH and the purified rmlA, rmlB, rmlC, and rmlD (Y4) gene products were added to the reaction mixture. F, NADH and the purified rmlA, rmlB, rmlC, and tll gene products were added to the reaction mixture.
Identification of Sugars in the Products by Enzymatic Reactions—
The D-((1)-2-octyl glycoside acetates derived from the sugar components of the dTDP-sugars were analyzed by gas-liquid chromatography (Fig. 4). The peaks of the dTDP-sugar derivatives produced by the putative dTDP-L-rhamnose-producing dTDP-6-deoxy-L-lyxo-4-hexulose reductases forming from strain Y4 and NCTC 9710 were in agreement with that of authentic L-rhamnose (Fig. 4, A, B, and C). Because authentic 6-deoxytalose is not commercially available, hydrolysates of serotype-specific polysaccharides purified from strain NCTC 9710 (serotype c) and ATCC 29523 (serotype a) were used as standards for 6-deoxy-L-talose and 6-deoxy-D-talose, respectively (2). (Fig. 4, D and F). The profile of a derivative of the dTDP-sugar synthesized by the tll gene product was in agreement with that of 6-deoxy-L-talose, a hydrolysate of the purified serotype c-specific antigen or 6-deoxy-L-talose. (Fig. 3F). The peaks of putative dTDP-L-rhamnose and dTDP-6-deoxy-L-talose were collected and analyzed by gas-liquid chromatography and NMR.

NMR Analysis of dTDP-L-rhamnose and dTDP-6-deoxy-L-talose—Approximately 2 mg of dTDP-6-deoxy-L-talose and dTDP-L-rhamnose were pooled from several HPLC runs on an ODS-80Ts (Fig. 3, D and F). After removing the excess phosphate by adding ethanol, the solution was concentrated by evaporation. The concentrated solutions were lyophilized and dissolved in D$_2$O. The NMR spectrum of authentic L-rhamnose was also measured (data not shown). The NMR spectra of authentic dTDP and these dTDP-hexoses are shown in Fig. 5. Assignment of these resonances was verified by two-dimensional homonuclear $^1$H COSY experiments (Fig. 6). Assigned chemical shifts and coupling constants are summarized in Table I. Signals for the nucleotide moieties in the dTDP-sugars were in good agreement with those of dTDP. Signals for the sugar moiety of dTDP-L-rhamnose were in good agreement with the reported chemical shift values of $\beta$-L-rhamnose (17) except for H1''. The large downfield shift (0.35 ppm) of the H1'' signal can be attributed to neighboring phosphate groups, which also affect the H2'' signal, thereby causing the poor resolution. The signal of H3' is rather small, perhaps because it is affected by decoupling of water nearby. The coupling constants also supported the orientations of H2'', H3'', H4'', and H5''' being equatorial, axial, axial, and axial, respectively, except for the J$_{1,2}$ value of 8.80 Hz. This value is inconsistent with the chemical shift values indicating the $\beta$ configuration, and it is reasonable to think that the neighboring phosphate groups...
also affect the coupling constant. Because 6-deoxy-1-talose is not commercially available, the values of chemical shifts and coupling constants were compared with those of dTDP-L-rhamnose. The chemical shifts were in good agreement with those of dTDP-L-rhamnose except for H3 and J3,5 values were small coupling constants in contrast to the dTDP-L-rhamnose values. These results also support this sugar nucleotide being dTDP-6-deoxy-L-talose.

DISCUSSION

We have previously cloned the gene cluster essential for the biosynthesis of serotype b-specific polysaccharide antigen from A. actinomyctemcomitans Y4 and identified the four genes coding for the enzymes that synthesize dTDP-1-rhamnose from D-glucose 1-phosphate, dTTP, and NADPH (10, 12). The genes coding for glucose-1-phosphate thymidylyltransferase, dTDP-D-glucose-4,6-dehydratase, dTDP-6-deoxy- D-xylo-4-hexulose-3,5-epimerase, and dTDP-6-deoxy- L-lyxo-4-hexulose reductase are designated as rmlA, rmlB, rmlC, and rmlD, respectively (18). The gene cluster essential for the biosynthesis of serotype c-specific polysaccharide antigen, 6-deoxy-1-talan, was also cloned from A. actinomyctemcomitans NCTC 9710; four genes whose products exhibited high homology to the rmlA, rmlB, rmlC, and rmlD gene products were found (11). The amino acid sequences of glucose-1-phosphate thymidylyltransferase, dTDP-D-glucose-4,6-dehydratase, and dTDP-6-deoxy-D-xylo-4-hexulose-3,5-epimerase of strain Y4 show more than 90% identity with their strain NCTC 9710 homologues, whereas strain Y4 dTDP-6-deoxy-L-lyxo-4-hexulose reductase shares only 58% identity with its corresponding gene product in strain NCTC 9710. We expected the rmlD homologue of strain NCTC 9710 to be the gene coding for dTDP-6-deoxy-L-lyxo-4-hexulose reductase, which synthesizes dTDP-6-deoxy-L-talose. We expected this because both dTDP-6-deoxy-L-talose and dTDP-L-rhamnose are predicted to be synthesized from dTDP-6-deoxy-L-lyxo-4-hexulose by reduction, and the stereoselectivity of the reduction determines the direction of synthesis of these compounds to dTDP-6-deoxyhexoses (11). Contrary to our expectations, the gene product of the rmlD homologue of strain NCTC 9710 converted dTDP-6-deoxy-L-lyxo-4-hexulose to dTDP-L-rhamnose (Fig. 3E).

In comparing the strain NCTC 9710 cluster essential for 6-deoxy-L-talose synthesis with the gene cluster producing serotype b-specific polysaccharide antigen, it was found that ORF7, ORF8, and ORF9 (Fig. 1) were specific to the former cluster. The amino acid sequence of ORF8 product exhibited weak identity (22.6%) with E. coli UDP-glucose-4-epimerase and a GXXGXXG motif of a NAD-binding domain is present at the N terminus of the ORF8 product (19). UDP-glucose-4-epimerase catalyzes both the conversion of UDP-galactose to UDP-glucose and the reverse reaction; the target of the reaction is the C-4 position of the nucleotide-activated hexoses. L-Rhamnose and 6-deoxy-1-talose differ in the stereochemistry of the C-4 carbon. As serotype c-specific polysaccharide antigen does not contain L-rhamnose, we expected that dTDP-L-rhamnose would serve as a precursor for the synthesis of dTDP-6-deoxy-L-talose and that the ORF8 product would be dTDP-L-rhamnose-4-epimerase for the conversion of dTDP-L-rhamnose to dTDP-6-deoxy-L-talose.

**TABLE I**

| Proton | dTTP | dTDP-L-rhamnose | dTDP-6-deoxy-L-talose |
|--------|-------|-----------------|-----------------------|
|        | δ (ppm) | δ (ppm) | δ (ppm) |
|        | Chemical shift | Coupling constant | Chemical shift |
| H-3    | 7.80(s) | 7.78(s) | 7.81(s) |
| H-6    | 7.77(s) | 7.74(s) | 7.76(s) |
| CH3    | 1.95(s) | 1.91(d) | 1.93(d) |
| H-1'   | 6.37(t) | 6.34(t) | 6.37(t) |
| H-2'   | 2.38(dd) | 2.35(dd) | 2.38(dd) |
| H-3'   | 4.65(m) | 4.61(dd) | 4.64(dd) |
| H-4'   | 4.19(dd) | 4.16(dd) | 4.19(dd) |
| H-5'   | 4.00(m) | 4.01(m) | 4.00(m) |
| H-1    | 5.21(d) | J1,2 8.80 | 5.17(d) |
| H-2    | 4.08(dd) | J2,3 2.92 | 4.06(dd) |
| H-3'   | 3.63(dd) | J3,4 9.76 | 3.82(dd) |
| H-4'   | 3.36(dd) | J4,5 9.28 | 3.65(dd) |
| H-5'   | 3.49(m) | 1.30(d) | 3.75(m) |

*a* singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet.

* indicates the signal is broad and weakly coupling with H-5.
Again contrary to our expectations, the ORF8 product did not convert dTDP-β-rhamnose to dTDP-6-deoxy-β-talose (data not shown), but dTDP-6-deoxy-β-lyxo-4-hexulose to dTDP-6-deoxy-β-talose (Fig. 3F). NMR and gas-liquid chromatography analyses also showed that the product of the reaction was dTDP-6-deoxy-β-talose and corresponded to the component of serotype c-specific polysaccharide antigen (Figs. 4 and 5). This gene product was therefore identified as a dTDP-6-deoxy-β-lyxo-4-hexulose reductase for synthesizing dTDP-6-deoxy-β-talose, and the gene was designated tll.

In fact, the formation of dTDP-6-deoxy-β-talose by the enzymatic activity of dTDP-6-deoxy-β-lyxo-4-hexulose reductase was detected in E. coli O45 27 years ago, but no characterization of the enzyme has since been reported (9). Bacterial cell surface polysaccharides consisting solely of 6-deoxy-β-talose are rare, although this hexose has been found as a component of some bacterial polysaccharides (5–7). The type II O-antigenic polysaccharide of Burkholderia pseudomallei lipopolysaccharide contains 6-deoxy-β-talose and the cluster of 15 genes required for its production has been identified and sequenced (20). The rml genes were also found in this gene cluster, although B. pseudomallei lipopolysaccharide does not contain β-rhamnose. Three genes, wbiB, wbiG, and wbiI, were viewed as candidates for nucleotide sugar epimerases involved in the conversion of dTDP-β-rhamnose to dTDP-6-deoxy-β-talose. None of these gene products shares homology with the tll gene product except for the consensus NAD-binding domains (GXXGXXG). In B. pseudomallei, dTDP-β-rhamnose may be a precursor of dTDP-6-deoxy-β-talose.

dTDP-6-deoxy-β-talose-forming dTDP-6-deoxy-β-lyxo-4-hexulose reductase shares the common substrate dTDP-6-deoxy-β-lyxo-4-hexulose with that forming dTDP-β-rhamnose, their

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Fig. 7. Alignment of dTDP-6-deoxy-β-lyxo-4-hexulose reductases. Tll, dTDP-6-deoxy-β-talose-forming dTDP-6-deoxy-β-lyxo-4-hexulose reductase from A. actinomycetemcomitans NCTC 9710; RmlD(c), dTDP-β-rhamnose-forming dTDP-6-deoxy-β-lyxo-4-hexulose reductase from A. actinomycetemcomitans NCTC 9710; RmlD(b), dTDP-β-rhamnose-forming dTDP-6-deoxy-β-lyxo-4-hexulose reductase from A. actinomycetemcomitans Y4. Asterisks above the sequences indicate the NAD-binding motif.

Fig. 8. Pathways for the synthesis of dTDP-6-deoxy-β-talose, dTDP-β-rhamnose, and dTDP-6-fucose from dTTP and β-glucose 1-phosphate in A. actinomycetemcomitans. The genes coding for the enzymes are indicated in parentheses.
only difference is stereoselectivity of the products. Nonetheless, they exhibited a very low degree (20.6%) of homology in their amino acid sequences (Fig. 7). A. actinomycetemcomitans Y4 dTDP-6-deoxy-d-xylo-4-hexulose reductase, responsible for forming dTDP-β-fucose, is also a dTDP-6-deoxy-4-hexulose reductase (12). The amino acid sequence of this reductase and that of dTDP-6-deoxy-l-talose-synthesizing dTDP-6-deoxy-l-lyxo-4-hexulose reductase show very low (15.3%) homology. It seems unsurprising that the homologies among the dTDP-6-deoxy-4-hexulose reductases are very low when compared with analogous enzymes of other organisms. CDP-abequose synthase, encoded by the abe gene in Salmonella enterica LT2 serovar Typhimurium, converts CDP-3,6-dideoxy-D-threose, encoded by the analogous enzymes of other organisms. CDP-abequose synthase, encoded by the per gene in S. enterica TY2, converts the same sugar nucleotide to CDP-3,6-dideoxy-d-glucose, CDP-abequose (21). CDP-paratose synthase, encoded by the abe gene in L. sake Y4 (serotype b) of dTDP-6-deoxy-D-fucose, is also a dTDP-6-deoxy-4-hexulose reductase-forming dTDP-6-deoxy-D-fucose, a biosynthetic pathway of unique dTDP-6-deoxyhexoses not previously found in other organisms was elucidated (Fig. 8).

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