Scission of the Lactyl Ether Bond of N-Acetylmuramic Acid by Escherichia coli “Etherase”*

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The ubiquitous bacterial cell wall sugar N-acetylmuramic acid (MurNAc) carries a unique C-3-lactyl ether substituent at the C3 position. Recently, we proposed an etherase capable of cleaving this lactyl ether to be part of the novel bacterial MurNAc dissimilation pathway (1). Here, we report the identification of the first known MurNAc etherase. The encoding gene murQ is located at 55 min on the Escherichia coli chromosome adjacent to murP, the MurNAc-specific phosphotransferase system. A murQ deletion mutant could not grow on MurNAc as the sole source of carbon and energy but could be complemented by expressing murQ from a plasmid. The mutant had no obvious phenotype when grown on different carbon sources but accumulated MurNAc 6-phosphate at millimolar concentrations from externally supplied MurNAc.

Purified MurQ-His6 fusion protein and extracts of cells expressing murQ both catalyze the cleavage of MurNAc 6-phosphate, with GlcNAc 6-phosphate and D-lactate being the primary products. The ¹⁸O label from enriched water is incorporated into the sugar molecule, showing that the C3–O bond is cleaved and reformed by the enzyme. Moreover, an intermediate was detected and identified as an unsaturated sugar molecule. Based on this observation, we suggested a lyase-type mechanism (β-elimination/hydration) for the cleavage of the lactyl ether bond of MurNAc 6-phosphate. Close homologs of murQ were found on the chromosome of several bacteria, and amino acid sequence similarity with the N-terminal domain of human glucokinase-regulatory protein (GckR or GKRP) was recognized.

The bacterial cell wall sugar N-acetylmuramic acid (MurNAc),† together with N-acetylglucosamine (GlcNAc), forms the backbone of the cell wall peptidoglycan of Gram-positive and Gram-negative bacteria (1). The bacterial peptidoglycan (murain) can be cleaved by peptidoglycan hydrolases (muramidases), such as lysozyme, (endo-) N-acetylmuramoylaminopeptidases, and amidases (2, 3); however, the fate of the monosaccharide MurNAc is unknown. MurNAc can be utilized by Escherichia coli (4, 5) and other bacteria (5) as the sole source of carbon, nitrogen, and energy. Recently, we identified a phosphotransferase system (PTS) of E. coli, MurP, that is required for phosphorylation and concomitant transport of MurNAc across the cytoplasmic membrane (5). Moreover, we recognized that the MurNAc dissimilation pathway enters the GlcNAc pathway on the level of GlcNAc 6-phosphate (Fig. 1) and thus involves the cleavage of the lactyl ether substituent of MurNAc 6-phosphate by a hypothetical “etherase” prior to deacetylation and deamination of the sugar (6). However, neither the lactyl ether-cleaving enzyme nor the second reaction product, other than GlcNAc 6-phosphate, have yet been identified.

The trivial name etherase implies catalysis by hydrolytic scission of the substrate; however, cleavage of ether bonds is catalyzed by a heterogeneous group of enzymes or enzyme systems exhibiting a variety of mechanisms, including oxidoreductase and lyase-type reactions (7). An example of an etherase that catalyzes the direct hydrolysis of a C–O bond similar to the MurNAc lactyl ether bond is the isochorismate pyruvate hydrolase (isochorismatase; E.C. 3.3.2.1) (8). This enzyme catalyzes the hydrolysis of a vinyl ether-functional group (an ether-bound enolpyruvate), yielding pyruvate as one of the products (9). This reaction resembles the reverse of the biosynthesis pathway in which UDP-MurNAc is formed from UDP-GlcNAc and phosphoenolpyruvate in a two-step process. In the first step, the transfer of enolpyruvate from phosphoenolpyruvate to position 3 of the GlcNAc residue is catalyzed by a transferase (MurA) to yield UDP-GlcNAc-enolpyruvate. In the second step, the reduction of the enolpyruvate moiety to D-lactyl is catalyzed by a reductase (MurR) to yield UDP-MurNAc (10). The only etherase cloned and sequenced so far is LigE, an enzyme from Pseudomonas putida F1 (9). This enzyme degrades low molecular weight lignin and catalyzes the reductive cleavage of arylglycerol-β-aryl ethers (12). Oxidoreductases that catalyze the oxidative cleavage of an ether bond are, for example, 4-methoxybenzoate monoxygenase and the glyceryl-ether monoxygenase (14, 15). The mechanism of anaerobic cleavage of 2-phenoxyethanol involves the shift of a hydroxyl group to the subterminal carbon atom to form an unstable hemiacetal (16, 17). Finally, carbon-oxygen lyases catalyze β-elimination reactions, with carboxy-methyl-oxysuccinate lyase (18) being one example.

Here, we identified the first lactyl etherase acting on the natural substrate MurNAc. It is encoded by murQ (previously yeF) located upstream of the gene murP encoding the MurNAc-PTS of E. coli. Purified MurQ protein releases D-lactate and GlcNAc 6-phosphate as the primary reaction products.

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‡ The abbreviations used are: MurNAc, N-acetylmuramic acid (2-acetamido-2-deoxy-ß-D-glucopyranose); MMA, minimal medium A; IPTG, isopropyl β-D-thiogalactopyranoside; ESI, electrospray ionization; PTS, phosphotransferase system; Km*, kana-mycin resistance cassette; SIS, sugar phosphate isomerase/sugar phosphate-binding protein.
FIG. 1. Dissimilation pathways of the amino sugars glucosamine (GlcN), N-acetylglosucamine (GlcNAc), and N-acetylmuramic acid (MurNAc) of E. coli. The amino sugars are transported across the cytoplasmic membrane and concomitantly phosphorylated by the phosphotransferase system (ManXYZ, NagE, and MurP). MurNAc 6-phosphate is processed by the etherase MurQ, identified in this work, to yield D-lactate and GlcNAc 6-phosphate, which is further degraded by the enzymes of the GlcNAc dissimilation pathway, NagA (the GlcNAc 6-phosphate deacetylase) and NagB (the GlcN 6-phosphate deaminase/isomerase), yielding fructose 6-phosphate, which enters glycolysis.

\[ ^{18}O \text{GlcNAc 6-phosphate was identified when the reaction was performed within } ^{18}O \text{water} \] and a Morgan-Elson chromogen was identified as the reaction intermediate. We proposed a lyase-type mechanism of catalysis that involves the formation of a $\Delta2,3$-GlcNAc 6-phosphate intermediate.

MATERIALS AND METHODS

Construction of Etherase Deletion Mutants—murQ (formerly named yfeL) of E. coli strain DY330 (F $\Delta$ [argF-lacU169 gal-490 (x1857 $\Delta$cro/braA)]) was deleted by replacement with a kanamycin resistance cassette (Km$^\text{r}$) according to a modification (5) of published protocols (19, 20). The following primers were used to amplify the Km$^\text{r}$ of plasmid pKD4 (20) in a PCR under conditions described elsewhere (5): 5'-TAG TAA GGT CAC CAC CGA TGG AAT TTG AAA AGA TGA TTA CTTATCC TCC TTA G-3' (underlined are the sequences complementary to the first and last 20 bp of the Km$^\text{r}$). The Km$^\text{r}$ was transferred by P1 transduction (21) into strain MC4100 (F $\Delta$ [argF-lacU169 blbB5301 araD139 deoC1 relA1 rbsrr psl150 ptsF25]) (22) yielding the deletion strain T2J (MC4100 $\text{Km} Q^\text{r}$).

Construction of Etherase Expression Vectors—DNA preparations, restriction enzyme digestions, ligations, and transformations were performed according to standard techniques. murQ from E. coli MG1655 was amplified by PCR using 50 ng of chromosomal DNA and the following primers: 5'-CAC CCA TGG AAT TTG AAA AGA TGA TTA C3', 5'-GAT CTC TTA AGC TTA AGA TTA TTC G-3' (underlined are the recognition sites for the restriction endonucleases NcoI, HindIII, and BglII, respectively). The reaction mixture was heated to 95 °C prior to the addition of 1 unit of Taq polymerase (Peqlab, Erlangen, Germany). Thirty-five cycles (30 s at 94 °C, 30 s at 57 °C, and 1 min at 72 °C) were performed in a thermal cycler and revealed a single 0.9-kb fragment, as analyzed by agarose gel electrophoresis. The amplified DNA was cloned into vector pCS19 (5), positioning the gene murQ under the control of the T5 promoter and the lac repressor. Digestion with NcoI and BglII and subsequent ligation generated plasmid pUB9 for the expression of the C-terminal His$_6$ fusion protein, and digestion with NcoI and HindIII and ligation generated pUB5 for the expression of the native protein.

Growth Conditions and MurNAc 6-Phosphate Accumulation—Strains MC4100 and T2J were cultivated in shaking flasks at 37 °C in minimal medium A (MMA) (21) supplemented with 0.2% MurNAc (6.8 mM) (Bachem, Bubendorf, Switzerland). For complementation studies, strain T2J was transformed with plasmids pUB5 or pUB9. Ampicillin (100 mg/liter) and isopropyl $\beta$-D-thiogalactopyranoside (IPTG) (0.05 mM) were added to the growth medium. Growth was monitored at 578 nm using an Ultraspec 3100pro spectrophotometer (Amersham Biosciences). For accumulation experiments, the strains were grown at 37 °C on MMA (10 ml) supplemented with 0.4% (43 mM) glycerol (Sigma) until they reached the logarithmic growth phase (A$_{600}$ = 0.5), and then 0.1% MurNAc (3.4 mM) was added and growth continued until the stationary phase. The cells were harvested by centrifugation (3000 $\times$ g for 15 min at 4 °C), resuspended in 15% trichloroacetic acid, and analyzed by thin-layer chromatography (TLC). MurNAc 6-phosphate was isolated from the TLC plates prior to charring by extracting scratched-out silica gel material with a small amount of distilled water.

Analysis of Sugars and Sugar Phosphates by TLC—Samples containing sugars and sugar phosphates were spotted onto TLC plates (Silica 60 F$_254$ (Merck, Darmstadt, Germany) and air dried. The following sugar standard solutions were used: 13.4 mM MurNAc 6-phosphate (10 μl), 72.7 mM GlcNAc 6-phosphate (1 μl), 77 mM glucosamine 6-phosphate (GlcN 6-phosphate; 1 μl), 82.8 mM MurNAc (10 μl), 928 mM GlcNAc (1 μl), and 904 mM GlcN (1 μl). The TLC was developed in two different, (i) a basic solvent system containing n-butyl alcohol:normethanol:ammonia:water (5:4:2:1) or (ii) an acid n-butyl alcohol:normethanol:5% formate (5:3:2) system. The TLC plates were air-dried and subsequently processed using 5% sulfuric acid in methanol and charred at 180 °C for 5 min.

Origin and Preparation of Sugars and Sugar Phosphates—MurNAc 6-phosphate probes for mass spectrometric analysis and for the enzymatic reaction assays were prepared from etherase mutant cells grown on MurNAc. The cells were incubated with trichloroacetic acid (15%) and kept on ice for 15 min. Insoluble material was removed by centrifugation (14,000 $\times$ g for 10 min), and the supernatant containing soluble material was applied to a TLC plate. The sugar phosphate was isolated from the TLC plate prior to charring by extracting scratched-out silica gel material with methanol. The extract was lyophilized and dissolved in a small amount of distilled water or $^{18}O$ water.

The MurNAc 6-phosphate standard was prepared previously from MurNAc, using membrane vesicles isolated from strains carrying the MurNAc-Pts PTS MurP, incubated together with purified soluble PTS components and phosphoenolpyruvate at pH 7.3 and 37 °C. MurNAc and muramic acid (MurN) were from Bachem (Bubendorf, Switzerland). AnhydroMurNAc was kindly provided by Dr. Niels Kubasch (Fachbereich Chemie, Lehrstuhl Prof. R. R. Schmidt, Universitat Konstanz, Germany). Other sugars and sugar phosphates were from Sigma.

Expression and Purification of Etherase—E. coli strain BL21 (F $ompF$ hsd S$_{r}$ (rB mB) gal dcm) carrying pUB9 was grown at 37 °C in LB with ampicillin (200 μg/ml). At an A$_{600}$ of 0.6, IPTG was added to a final concentration of 0.05 mM, and incubation was continued for 3 h with shaking. Cells were harvested by centrifugation (4,000 $\times$ g for 20 min at 4 °C) and resuspended in 10 ml of buffer (20 mM sodium phosphate, 10 mM imidazole, pH 8.0, and 5 mM 2-mercaptoethanol) to prevent oxidation of cysteines of the enzyme). The cells were broken by passing them three times through a French pressure cell. Debris and unbroken cells were removed by centrifugation at 27,000 $\times$ g for 30 min. The
MurQ Is Required for Growth on MurNAc—murQ, the gene adjacent to murP (encoding the MurNAc-specific phospho-transferase system [4]), was deleted by homologous gene replacement, yielding strain TJ2. No growth of TJ2 on MurNAc, as the sole source of carbon and energy, was detected even after 40 h of incubation (Fig. 2). The growth defect was not due to a polar effect of the murQ deletion on murP, because murP provided from a plasmid (5) did not restore growth (data not shown). However, providing MurQ or MurQ-His6 fusion protein or with soluble extracts from TJ2 cells expressing murQ from a plasmid. The first reaction product, GlcNAc 6-phosphate, was analyzed by TLC and mass spectrometry. The reaction was performed in H2O and [18O]water to identify a product carrying the 18O label by a shift in mass.

The second product of the etherase reaction, lactate, was analyzed by a coupled enzyme assay with either d- or l-lactate dehydrogenase (Roche Applied Science). The amount of NADH formed in the coupled enzyme assay was determined by measuring the absorption at 340 nm. To shift the equilibrium of the coupled enzyme reaction to the product side, pyruvate was trapped in a subsequent reaction in the presence of l-glutamate by glutamate-pyruvate transaminase.

Analysis of the Reaction Intermediate—A reaction intermediate was identified with Ehrlich’s reagent (dimethylaminobenzaldehyde) in a color reaction according to the modified Morgan-Elson protocol (23). Instability of the lactate, which induces base-catalyzed formation of the chromogen, etherase preparations were added in brief. 2 μl of enzyme was added to 10 μl of sugar phosphate (GlcNAc 6-phosphate, MurNAc 6-phosphate; 10–100 mM) and incubated for 5–30 min at 37 °C. Subsequently, 60 μl of Ehrlich’s reagent was added, and the mixture was incubated for 20 min at 37 °C.

Mass Spectrometry Analysis—Liquid chromatography-tandem triple quadrupole mass spectrometry was carried out on an Alliance HT model 2695 liquid chromatography system (Waters Corp.) coupled to a Micromass QUATTRO mass spectrometry system (Micromass, Beverly, MA) supplied with MassLynx NT version 4.0 software (Micromass). Following a 20-μl injection, the sample was eluted at 250 μl/min. Mobile phase A was 99.9% water and 0.1% formic acid, and mobile phase B was 99.9% acetonitrile and 0.1% formic acid. Separation was achieved with the tandem coupling of a Phenomenex Hyperclone C18 (100×4.61-B0) column (150 × 2 mm; 5 μm) (Phenomenex, Torrence, CA). Eluent from the liquid chromatography system was analyzed using an electrospray ionization (ESI) technique. Sample solutions were introduced into the ion source at the flow rate of 250 μl/min via a metal capillary held at high voltage (1.8 kV). The source block temperature was 150 °C, and the desolvation temperature was 250 °C; the desolvation gas was set at 400 liters/h. Nitrogen was used as both a nebulizing gas and drying gas. The instrument was operated in the negative ion mode, which typically reports the characteristic appearance of [M-H] ions of acidic saccharides that exist in anionic form in solutions.

RESULTS

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It should be noted that, in contrast to wild type E. coli (MC4100), no lag phase was observed for the growth of TJ2 (murQ::Kmr) on MurNAc when complemented with MurQ (Fig. 2). Slightly higher growth rates were also seen in the complemented strain compared with wild type E. coli. This observation can be explained by low level constitutive expression of murQ from the promoter of the kanamycin resistance gene (Kmr replaces murQ), which is located upstream in TJ2. Therefore, the MurNAc-PTS does not require induction in TJ2, but it does in wild type E. coli.

MurQ Requires Reducing Conditions for Activity and Stability—Purification of active etherase was complicated by the fact that this enzyme contains seven cysteine residues that make it extremely sensitive to oxidation. Therefore, 5 mM 2-mercaptoethanol was added to the cells before disruption and to buffers for nickel affinity chromatography. Purified etherase was only active for a few days at 4 °C. However, the enzyme could be stabilized in imidazole buffer (pH 8.0) by adding 20% glycerol and 1 mM dithiothreitol, and activity was retained for at least several weeks when stored at −20 °C. It is unclear whether the cysteines are essential for enzyme function.

A murQ Mutant Accumulates MurNAc 6-Phosphate—Besides the growth defect on minimal medium MurNAc, no obvious phenotype was observed when the MurQ knock-out strain was grown on other carbon sources in minimal or rich medium. However, TJ2 (murQ::Kmr) cells (but not wild type E. coli cells) grown on glycerol in MMA accumulated a metabolite when MurNAc was added to the cells. This accumulation product was identified with Ehrlich’s reagent (dimethylaminobenzaldehyde) in a color reaction according to the modified Morgan-Elson protocol (23). Instability of the lactate, which induces base-catalyzed formation of the chromogen, etherase preparations were added in brief. 2 μl of enzyme was added to 10 μl of sugar phosphate (GlcNAc 6-phosphate, MurNAc 6-phosphate; 10–100 mM) and incubated for 5–30 min at 37 °C. Subsequently, 60 μl of Ehrlich’s reagent was added, and the mixture was incubated for 20 min at 37 °C.

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MurNAc 6-phosphate accumulation analyzed by TLC. Sugars and sugar phosphates extracted from MC4100 (wild type) and TJ2 (murQ::kan) cells grown in MMA containing 0.4% (43 mM) glycerol without lanes 1 and 3) and with lanes 2 and 4) the addition of 0.1% (3.4 mM) MurNAc. MurNAc 6-phosphate accumulation was visible in cell extracts of strain TJ2 only when MurNAc was added to the culture (lane 4). The TLC was developed using method 1. The following standards were spotted: MurNAc 6-phosphate (MurNAc 6P), GlcNAc 6-phosphate (GlcNAc 6P), MurNAc, and GlcNAc. The concentration of accumulated MurNAc 6-phosphate in MurQ mutant cells supplied with MurNAc was calculated to be in the range of 30 mM. Interestingly, accumulation of MurNAc 6-phosphate did not affect growth on glycerol (data not shown). In general, sugar phosphate accumulation has been shown to severely affect growth; e.g. GlcNAc 6-phosphate accumulation in a nagB strain (nagB polar on nagA) in the presence of GlcNAc in the medium has been reported to lead to growth arrest (24). It is not clear why, in the case of MurNAc 6-phosphate, accumulation seems not to be toxic for the cells.

MurQ Converts MurNAc 6-Phosphate into GlcNAc 6-Phosphate and D-Lactate—Cell extracts containing overexpressed MurQ and purified MurQ-His6 protein cleave MurNAc 6-phosphate, yielding GlcNAc 6-phosphate, as analyzed by TLC (Fig. 5) and by ESI-mass spectrometry (Fig. 6B). A mass value of 299.83 was found, which is in good agreement with the mass of GlcNAc 6-phosphate (300.06). Performing the etherase reaction in [18O]water led to the identification of an 18O-labeled GlcNAc 6-phosphate (300.06). Performing the etherase reaction, indicating the reversibility of the enzymatic reaction. Based on the color reaction, we are now developing an etherase assay to perform detailed kinetic measurements.

MurQ Homologs Are Found in Many Bacteria; However Not in All—MurQ homologs were found on the chromosome of other enterobacteria and in related γ-proteobacteria of the genera Vibrio and Pasteurella, as well as in cyanobacteria. MurQ homologs were, however, not found in Pseudomonas sp. and β-proteobacteria. Interestingly, close homologs of MurQ were also found in many Gram-positive bacteria, in members of the genera Bacillus, Lactobacillus, Clostridia, and Actinobacillus. The MurNAc dissimilation might therefore be a universal pathway of many bacteria (but not all), and MurNAc dissimilation might be used as a criteria for the taxonomy of bacteria.

**DISCUSSION**

We describe in this paper the identification of an etherase (MurQ) that catalyzes the cleavage of the β-lactyl ether substrate of MurNAc 6-phosphate, representing a novel class of etherase enzymes. Active His6-tagged fusion protein was purified to homogeneity and was shown by TLC, mass spectrometry, and coupled enzyme assay to yield GlcNAc 6-phosphate and β-lactate. When the reaction was performed in [18O]water, the label was incorporated in the product GlcNAc phosphate and not lactate, indicating that the C3-O bond is cleaved and reformed by the enzyme.

Usually, ether linkages are comparably resistant to chemical or enzymatic cleavage, because the C-O bond energy is high (25). However, the lactyl ether bond of MurNAc and its derivatives, in which reducing groups are free, can be cleaved spontaneously under mild alkaline conditions (26). The H2 proton in the α position relative to the anomeric carbonyl is acidic. Deprotonation and subsequent elimination of the C3 substituent leads to the formation of a 2,3-unsaturated sugar, Δ2,3-GlcNAc. This intermediate is the so-called Morgan-Elson chromogen I, which reacts further to generate an aromatic furan derivative, the Morgan-Elson chromogen III (26). That chromogen can be identified through reaction with Ehrlich’s reagent, which yields a purple-colored Morgan-Elson product (23).
We identified a Morgan-Elson chromogen being formed by the enzyme proceeding in both the forward and reverse directions. This reaction intermediate has an absorption maximum of ~235 nm, consistent with the absorption maximum of Morgan-Elson chromogen I. It decolorized bromine water (data not shown) and reacted with Ehrlich’s reagent. Based on this finding, we expect MurQ to catalyze a two-step β-elimination/hydration reaction. A high steady-state level of the intermediate is formed, most likely Δ2,3-GlcNAc 6-phosphate, indicating that hydration is the rate-limiting step of the reaction.
According to the suggested mechanism (Fig. 7), a catalytic base provided by the enzyme removes the H2 proton of MurNAc 6-phosphate. Subsequent elimination of the lactyl substituent with retention of the D-configuration leads to the formation of the Δ2,3-GlcNAc intermediate (Morgan-Elson chromogen I). Subsequent addition of water to the double bond of the intermediate (a Michael addition acceptor) finally yields GlcNAc 6-phosphate. Performing the reaction in [18O]water led to the identification of 18O-labeled GlcNAc-6-phosphate (Fig. 7). It is obvious that the H2 proton of MurNAc 6-phosphate is only acidic when the sugar carries a reducing end and is able to adopt an open chain configuration. It is therefore not surprising that anhydro-MurNAc cannot act as a substrate in the MurQ reaction.

A similar mechanism that can be viewed as a mirror image of the etherase mechanism has recently been proposed for members of family 4 of glycosyl hydrolases (GH-F4) (27–29). These enzymes act on glycosides, hence the anomeric carbonyl is not only acidic when the sugar carries a reducing end and is able to adopt an open chain configuration. It is therefore not surprising that anhydro-MurNAc cannot act as a substrate in the MurQ reaction.

MurQ can be assigned to the sugar phosphate isomerase/sugar phosphate-binding protein (SIS) family (30). Sequence comparison of MurQ with the E. coli chromosome shows high similarity with the SIS domain of GlmS, the glucosamine-fructose 6-phosphate (isomizerizing) aminotransferase (31). Besides the SIS domain, GlmS also contains a glutaminase domain. Ammonia generated by the glutaminase domain serves to form a Schiff base with C2 of fructose 6-phosphate (C–N lyase mechanism), and the Schiff base is then isomerized to glucosamine 6-phosphate. Moreover, MurQ is homologous to another member of the SIS family, the fructoselysine 6-phosphate deglycase, FrlB (YhfN) (32). FrlB catalyzes the reversible conversion of fructoselysine 6-phosphate and water to glucose 6-phosphate and lysine. A mechanism similar to that of GlmS was suggested (32): the C–N bond between the sugar phosphate molecule (fructose 6-phosphate) and the amino acid lysine is cleaved (C–N lyase reaction), and the product is then isomerized, yielding glucose 6-phosphate. Hence, GlmS and FrlB are both C–N lyases and characterized by a mechanism similar to that proposed for MurQ (Fig. 7). However, it is not clear whether the etherase reaction also depends on a Schiff base being formed.

Interestingly, MurQ displays high amino acid sequence similarity (26% identity) with the N-terminal domain of human glucokinase regulatory protein (GckR or GKRP), another member of the SIS family (33). In vivo GckR reversibly binds to glucokinase (hexokinase IV) and competitively inhibits its activity. The regulatory protein exists in two different conformations: one (R) able to bind fructose 6-phosphate and glucokinase and the other (R') able to bind fructose 1-phosphate but not glucokinase. In the absence of metabolite, the R' form predominates and glucokinase is active. Fructose 6-phosphate shifts the equilibrium toward R, hence reinforcing inhibition. Fructose 1-phosphate sequesters the R' form and has therefore the opposite effect. It is not known whether the sequence similarity between MurQ and GckR just reflects sugar phosphate binding or whether the lyase reaction of MurQ has some equivalence in the human protein.

We found that MurQ is essential for growth on MurNAc, as the sole source of carbon and energy. However, E. coli is able to re-utilize its own cell wall in addition to dissimilating exogenous MurNAc. In this process, 1,6-anhydro-N-acetyl muramic acid (anhydro-MurNAc) is formed within the cytoplasm. The further processing of anhydro-MurNAc had been postulated to proceed through an etherase that generates GlcNAc from anhydro-MurNAc (34). Recently, a kinase encoded by gene ydhH at 37 min on the E. coli chromosome has been identified that phosphorylates anhydro-MurNAc, presumably at the C6 position (35). Hence, cell wall recycling and dissimilation of MurNAc from the environment yield the same metabolite, MurNAc 6-phosphate, which might be further metabolized by the same etherase enzyme. The murQ deletion strain (TJ2) did not grow on MurNAc, even when incubated for prolonged times, indicating that MurQ is the only lactyl etherase of E. coli. Therefore, it will be interesting to investigate the role of MurQ in cell wall recycling. We have not detected MurNAc 6-phosphate in etherase mutant cells without the addition of MurNAc to the medium. However, this might be explained by the limited sensitivity of detection of MurNAc 6-phosphate with the TLC method. It was estimated that intracellular concentrations of MurNAc 6-phosphate <2 mM cannot be detected with the TLC method, which might be too high a value to reach within the cells solely through the recycling of cell wall murein. We are currently studying the role of MurQ in cell wall recycling, and we are trying to obtain a three-dimensional structure with the aim of

**TABLE I**

| Substrate | Concentration g/liter | l-Lactate g/liter | d-Lactate g/liter |
|-----------|-----------------------|------------------|------------------|
| d-Lactate | 0.202                 | 0                | 0.184            |
| l-Lactate | 0.202                 | 0.194            | 0                |
| MurNAc 6P | ND                    | 0                | 0.117            |
| MurNAc 6P | ND                    | 0                | 0.198            |

*a Substrate generated by in vitro phosphorylation.

*b Substrate generated by accumulation in TJ2 cells.

*c ND, not determined.

**FIG. 7. Proposed mechanism of the MurQ-catalyzed lactyl ether cleavage.** The first half-reaction involves the deprotonation of the acidic H2 proton, α-positioned to the C1 carbonyl, which is facilitated by an enzymic base (enzymic A–H). β-Elimination of d-lactate, possibly facilitated by a enzymic acid/base (enzymic B), leads to the formation of the Δ2,3-GlcNAc intermediate (Morgan-Elson chromogen I). In the second half-reaction, the addition of water ([18O]water) to the elimination product generates the second reaction product, GlcNAc 6P, carrying the 18O label.
obtaining a detailed understanding of the etherase mechanism on the molecular level.

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