JOURNAL OF BACTERIOLOGY, Feb. 2006, p. 1660–1662 Vol. 188, No. 4
0021-9193/06/$08.00+0 doi:10.1128/JB.188.4.1660–1662.2006

MurQ Etherase Is Required by *Escherichia coli* in Order To Metabolize Anhydro-*N*-Acetylmuramic Acid Obtained either from the Environment or from Its Own Cell Wall

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Received 11 August 2005/Accepted 30 November 2005

MurQ is an *N*-acetylmuramic acid-phosphate (MurNAc-P) etherase that converts MurNAc-P to *N*-acetylglucosamine-phosphate and is essential for growth on MurNAc as the sole source of carbon (T. Jaegar, M. Arsic, and C. Mayer, J. Biol. Chem. 280:30100–30106, 2005). Here we show that MurQ is the only MurNAc-P etherase in *Escherichia coli* and that MurQ and AnmK kinase are required for utilization of anhydro-MurNAc derived either from cell wall murein or imported from the medium.

The bacterial exoskeleton, i.e., the murein sacculus, is dynamic during growth. In *Escherichia coli*, an estimated two-thirds of the murein of the side wall is degraded each generation (5, 8) by lytic transglycosylases and endopeptidases (6). The principal degradation product is the anhydro-muropeptide, *N*-acetylglucosamine (GlcNAc)-1,6-anhydro-MurNAc (anhMurNAc)-L-Ala-γ-D-Glu-*meso*-diaminopimelic acid-D-Ala (6). The anhydro-muropeptide(s) is transported by AmpG permease to the cytoplasm, where it is degraded by AmpD, an anhMurNAc-L-Ala amidase; NagZ, a β-*N*-acetylglucosaminidase; and LdcA, an L,D-carboxypeptidase to release GlcNAc, anhMurNAc, murein tripeptide, and D-Ala (1, 2, 7–9, 15, 20). Most of the murein tripeptide directly enters the murein biosynthesis pathway (11), but it can also be degraded to the individual amino acids (14, 16). GlcNAc and anhMurNAc are also reused (13). Figure 1 shows the recycling pathway for the two amino sugars. GlcNAc is phosphorylated by a specific kinase, NagK, to generate GlcNAc-6-P, which can be readily metabolized by the well-known GlcNAc pathway (17). We have recently shown that for anhMurNAc to be recycled it is first phosphorylated with the simultaneous cleavage of the 1,6-anhydro ring by a newly identified kinase, AnmK, to generate MurNAc-P, which is converted to GlcNAc-P by what was then an unidentified etherase (18).

Very recently, a MurNAc-6-P etherase, named MurQ, has been identified which is required for growth of *E. coli* on MurNAc as the sole source of carbon (10). MurNAc is imported by a phosphotransferase system that requires a newly identified component, MurP, to yield cytoplasmic MurNAc-6-P (3). MurQ then hydrolyzes the ether bond between the hexose backbone and D-lactic acid moieties of MurNAc-6-P to produce GlcNAc-6-P and D-lactic acid (10). It was shown that MurNAc-6-P accumulates in *murQ* mutant cells incubated in the presence of MurNAc (10). These results suggest that the pathway to utilize anhMurNAc derived from murein and the pathway to metabolize MurNAc from the medium may merge at the stage of formation of MurNAc-6-P. If so, one would predict that radioactive MurNAc-P would accumulate in a *murQ* mutant whose murein amino sugars are radioactively labeled, but not in a *murQ anmK* double mutant since it lacks the kinase to phosphorylate anhMurNAc. To test this under efficient labeling conditions, TP71BQ, a Δ*murQ nagB*::*kan* mutant of TP71 [F$^-$ *lysA opp araD139 rpsL150 deoC1 ptsF25 ftbB5301 rbsR relA1* Δ(*argF-lac*)] (8), and TP71BQanmK, an *anmK*::*cat* mutant of TP71BQ, were constructed. *murQ*::kan from the TJ2 strain (10) was first transduced into TP71 by T4gt7 phage (12), and the Kan^r cassette was removed by transformation of pCP20 plasmid carrying FLP recombinase as described before (4), followed by the transduction of *nagB*::*kan* (13) into the strain, to yield TP71BQ. TP71BQanmK was generated by transduction of *anmK*::*cat* (18) into TP71BQ. A cell extract of TP71BQ grown at 37°C overnight in LB medium totally lacked the MurNAc-P etherase activity, as determined by the etherase assay described previously (18; data not shown), indicating that *E. coli* expresses only one MurNAc-P etherase. The existence of a single etherase in *E.* coli was indicated previously from the observation that a *murQ* mutant was unable to grow on MurNAc as the sole source of carbon (10).

The *murQ* mutant and the *murQ anmK* mutant were labeled with [6-^{3}H]glucosamine (GlcN) (21.6 Ci/mol; NEN Life Science Products, Boston, MA) in a glycerol M9 minimal medium (18), and the hot water extracts from cells at mid-log phase were analyzed by high-pressure liquid chromatography as described previously (16, 18). GlcN in the medium is taken up and phosphorylated by a phosphotransferase system, ManXYZ, to generate GlcN-6-P, which is converted to UDP-GlcNAc, a precursor of murein amino sugars and outer membrane lipopolysaccharide. The amounts of radioactivity in anhMurNAc and UDP-MurNAc-pentapeptide in extracts from TP71BQ and TP71BQanmK were similar (data not shown). However, the column flowthrough (FT) of the *murQ* mutant contained over six times as much radioactivity as that of the *murQ anmK* mutant (Table 1). Since anhMurNAc is known to escape into the medium from an *anmK* mutant (18),

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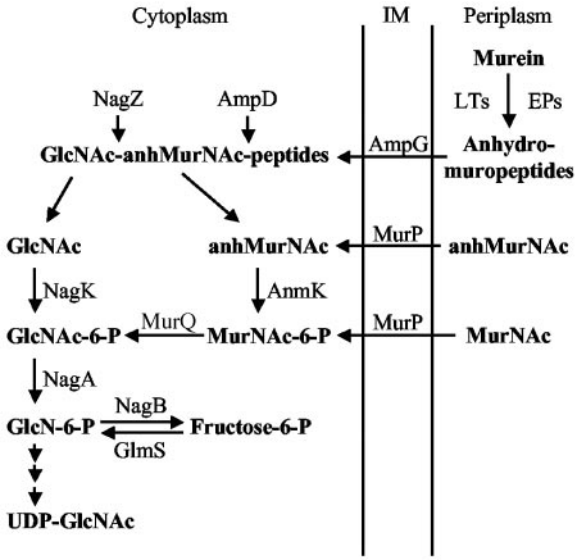

FIG. 1. Scheme for recycling of murein amino sugars and metabolism of exogenous anhMurNAc and MurNAc (3, 10, 18). IM, inner membrane; EPs, endopeptidases; LTs, lytic transglycosylases.

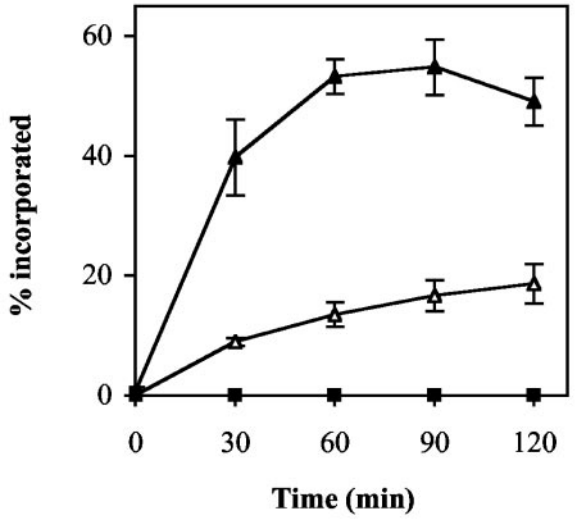

FIG. 2. Time course of anhMurNAc uptake by cells. anhMurNAc (1,000 cpm) was incubated in 10 μl of LB medium with cells from 1 ml of overnight culture. Cells were separated by centrifugation (12,000 × *g*, 1 min, 4°C) and washed three times with saline, and the amount of radioactivity in the supernatant and cells was measured. Open triangles, TP71B; closed triangles, TP71BQ; close squares, TP71BQanmK.

the lack of accumulation of label in the TP71BQanmK cell extract was expected. The FTs were analyzed by thin-layer chromatography (TLC) in an acidic solvent, *n*-butanol–acetic acid–water (4:1:1), following treatment with calf intestinal phosphatase as described previously (18). Eighty-six percent of the radioactivity in the dephosphorylated FT from the *murQ* mutant had the same R_f as MurNAc, indicating that MurNAc-P was the principle radioactive compound in the FT. The double mutant lacked radioactivity at this R_f, demonstrating that AnmK was required to trap MurNAc in the phosphorylated form (Table 1). When the FT from the *murQ* mutant was incubated at 37°C for 30 min with the *E. coli* etherase purified partially as described in reference 18 followed by the dephosphorylation with calf intestinal phosphatase and analysis by TLC with the acidic solvent, the amount of radioactivity at the same R_f as MurNAc decreased by 83% and instead the amount at the same R_f as GlcNAc increased fourfold, demonstrating that the main compound in the FT from the *murQ* mutant was converted to GlcNAc-P by the *E. coli* etherase. These results indicate that the principal radioactive compound derived from the murein sacculus present in the cytoplasm of the *murQ* mutant is MurNAc-P. This conclusion appears to contradict the previous report that accumulation of MurNAc-P was not detected in *murQ* cells grown without MurNAc (10). However, the concentration of MurNAc-P in the *murQ* mutant grown in the absence of MurNAc can be calculated to be 7 mM relative to a reported concentration of 0.175 mM UDP-MurNAc-pentapeptide in the cytoplasm (19). The amount analyzed, from 10 ml of culture containing 7 mM MurNAc-P, is just at the limit of detection by the method employed (10). Hence, the accumulation of MurNAc-P in a *murQ* mutant grown in the absence of MurNAc indicates that MurQ is required in order for *E. coli* to metabolize anhMurNAc.

TABLE 1. Effects of *murQ* and *murQ anmK* mutations on distribution of ^{3}H-sugars in hot water extracts of cells labeled with [6-^{3}H]GlcN[a]

Compound	Total counts (kcpm)[b]	
	murQ	*murQ anmK*
HPLC FT	154	24
GlcNAc	5	5
GlcNAc-P	15	16
MurNAc-P	132	0
UDP-GlcNAc	2	2
UDP-MurNAc-pentapeptide	3	4

[a] Amino sugars were isolated from 4 ml of cell culture grown in glycerol minimal medium containing 1 μCi of [6-^{3}H]GlcN/ml.
[b] Values were normalized to the turbidity of the cell culture (100 Klett units).

Since *E. coli* can use MurNAc as the sole source of carbon (3), it was of interest to examine whether anhMurNAc can be taken up by *E. coli* and serve as a carbon source. Radioactive anhMurNAc was incubated in 10 μl of LB medium at 37°C over 120 min with the cells from 1 ml of overnight culture of *E. coli* TP71B, TP71BQ, or TP71BQanmK. As shown in Fig. 2, anhMurNAc was imported by TP71B and TP71BQ cells, but not by TP71BQanmK cells. The initial rate of uptake of anhMurNAc by TP71BQ was at least four times higher than that by TP71B, suggesting that the uptake system for anhMurNAc is expressed more in TP71BQ than in TP71B. When the hot water extracts of the *murQ* mutant cells and its parent cells incubated with radioactive anhMurNAc were analyzed by TLC with basic and acidic solvent systems combined with a phosphatase as described previously (18), MurNAc-P was the only radioactive compound present in the *murQ* cells, while the parent cells contained radioactive GlcNAc-P, GlcN-P, and UDP-GlcNAc, as expected for cells metabolizing MurNAc-P (see Fig. 1).

Since this result indicates that anhMurNAc is taken up by *E. coli*, the question arises of whether MurP is involved. Several

TABLE 2. Requirement of MurP for uptake of anhMurNAc

Strain tested	Uptake (%)
TP71B	23
TP71BmurP	3
TP71BmurP/pCS19	3
TP71BmurP/pCS19murP	39
TP71BQanmK/pCS19	0
TP71BQanmK/pCS19murP	0

[a] anhMurNAc (1,000 cpm) was incubated with cells from 1 ml of overnight culture in 10 μl of L broth for 1 h at 37°C.

lines of evidence suggest that MurP is required. For instance, 2 mM MurNAc completely inhibited the uptake of anhMurNAc (approximately 5 μM) by both TP71B and TP71BQ cells. Furthermore, TP71BmurP (Δ*murP nagB*::Km mutant of TP71) did not import anhMurNAc, and this defect was complemented by a plasmid carrying the *murP* gene (Table 2). Importation of anhMurNAc by TP71BQanmK cells was also defective. However, it was not restored by the plasmid expressing MurP (Table 2). From these results, we can conclude that anhMurNAc from the medium enters the cytoplasm via MurP, where it is phosphorylated and phosphorylation of the imported anhMurNAc requires AnmK. Since an efflux pump for anhMurNAc appears to exist (18), anhMurNAc taken up by cells is likely to be immediately exported to the medium if it is not phosphorylated by AnmK. MurP phosphorylates carbon 6 of MurNAc during its uptake (3), but cannot phosphorylate carbon 6 of anhMurNAc, probably because of the 1,6-anhydro ring.

We have identified the pathway by which *E. coli* can utilize anhMurNAc from the medium as shown in Fig. 1. Hence, *E. coli* was expected to grow on anhMurNAc as a sole source of carbon. However, in preliminary growth tests, *E. coli* wild-type strain TP71 did not grow in M9 minimal medium containing 0.27% anhMurNAc as the sole source of carbon, whereas it could grow on MurNAc. This suggests that anhMurNAc may be toxic to the cells.

The results of this study support the following conclusions. (i) MurQ is the only MurNAc-P etherase in *E. coli*. (ii) In the absence of MurQ etherase, MurNAc-P accumulates in cells provided AnmK kinase is present. (iii) Just as for utilization of anhMurNAc derived from the cell's murein, anhMurNAc acquired from the medium is first converted to MurNAc-P by the AnmK kinase followed by conversion to GlcNAc-P by the "etherase," MurQ. (iv) anhMurNAc is taken up by MurP. (v) AnmK kinase is needed to convert imported anhMurNAc to MurNAc-P. (vi) When AnmK is inactivated, anhMurNAc is returned to the medium as rapidly as it is taken up.

This work was supported in part by Public Health Service grant GM51610 from the National Institute of General Medical Sciences.

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