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Two Distinct Transpeptidation Reactions during Murein Synthesis in *Escherichia coli*

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Murein synthesized in ether-permeabilized cells of *Escherichia coli* deficient in individual penicillin-binding proteins (PBPs) and in the presence of certain β-lactam antibiotics was analyzed by high-pressure liquid chromatography separation of the muramidase split products. PBP 1b was found to be the major murein synthesizing activity that was poorly compensated for by PBP 1a. A PBP 2 mutant as well as mecillinam-inhibited cells showed increased activity in the formation of oligomeric muropeptides as well as UDP-muramylpeptidyl-linked muropeptides, the reaction products of transpeptidation, bypassing the lipid intermediate. In contrast, penicillin G and furazlocillin severely inhibited these reactions but stimulated normal dimer production. It is concluded that two distinct transpeptidases exist in *E. coli*: one, highly sensitive to penicillin G and furazlocillin, catalyzes the formation of hyper-cross-linked muropeptides, and a second one, quite resistant to these antibiotics, synthesizes muropeptide dimers.

Enlargement and division of the rod-shaped bacterium *Escherichia coli* represent two different processes which can be uncoupled to a certain extent by interfering with murein synthesis (4, 17, 18). Low concentrations of penicillin G specifically inhibit cell division without affecting cell elongation, causing filamentation of the cell (16). The amidinopenicillanic acid mecillinam, on the other hand, which causes *E. coli* to grow as an osmotically stable sphere (14), may be considered a specific inhibitor of the cell elongation system (4, 17, 18). It has therefore been proposed that cell elongation and division are catalyzed by different sets of enzymes (6, 16), although some enzymes may function during both processes. Two proteins which bind penicillin seem to be specifically involved in these processes: penicillin-binding protein 2 (PBP 2) is required for cell elongation, whereas PBP 3 is needed for cell division (1, 17, 18). The precise enzymatic specificity of these two proteins (5, 6, 15), however, is still a matter of debate.

The results presented here indicate the presence of two different transpeptidation reactions which tentatively can be assigned to the two enzyme systems responsible for cell elongation and cell division.

MATERIALS AND METHODS

Strains and growth conditions. All strains used in this study were derived from *E. coli* K-12 and are shown in Table 1. Cultures were grown routinely with shaking at 30°C in Penassay Broth (Difco Laboratories) supplemented with lipoic acid (1 μg/ml) for the growth of SP5003, with spectinomycin (30 μg/ml) for SP1026, with kanamycin (25 μg/ml) for SP1028, and with L-lysine and *meso*-DL-diaminopimelic acid (20 μg/ml each) for mutants DL64 and JE5684.

Murein synthesis in ether-permeabilized cells. In vitro murein synthesis was studied in ether-permeabilized cells (9) as described previously (8). Ether-treated cells (15 mg/ml) were incubated at 30°C for 1 h in a total volume of 200 μl in the presence of UDP-*N*-acetylmuramylpentapeptide (8 nmol) and 0.8 nmol of UDP-*N*-acetyl-[^{14}C]glucosamine (261 mCi/nmol; Amersham Corp.) in 0.05 M Tris hydrochloride buffer (pH 8.3) containing 0.05 M NH_4Cl, 0.02 M $MgCl_2$, and 0.001 M β-mercaptoethanol. Murein was collected on membrane filters and digested with *Chalaropsis* muramidase as described (8).

Muropeptide separation by HPLC. The muramidase digest products were reduced with sodium borohydride and separated by reverse-phase high-pressure liquid chromatography (HPLC) on a prepacked column (250 by 4 mm) with Shandon Hypersil ODS-18 as described previously (3). The muropeptides were eluted at 53°C with a linear gradient of 0.05 M sodium phosphate buffer, pH 4.31, to 0.075 M sodium phosphate buffer, pH 4.95, containing 15% methanol in 125 min at a flow rate of 0.4 ml/min. The radioactivity in the fractions (80 μl) was determined in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

Murein synthesis in ether-permeabilized mutants with PBP defects. In an attempt to characterize the specific role of the

TABLE 1. *E. coli* strains

Strain	Description	PBP defect	Source	Reference
KN126	F$^-$ *trpE*(Am) *tyr*(Am) *ilv supD*(Ts)	None	Spratt	12
SP6	As KN126, but *pbpA6 mrcA6*	1a, 2	Spratt	17
SP1026	*his supF* Δ*mrcB* Spc^r	1b	Spratt	2
SP1028	*thr leu thi supE lacY* Δ*mrcA* Kan^r	1a	Spratt	23
SP5003	*his supF srl*::Tn*10 recA56* Δ(*rodA-dacA-lip*) (λ d *rodA1*)	5	Spratt	19
PA3092	F$^-$ *thr leu thi his argH trp thyA lacY xyl mel fhuA supE rpsL*	None	Hirota	20
JE5684	F$^-$ *dap lys lac rpsE mtl xyl malA dacB12 dacA1191*	4, 5	Hirota	20
DL64	*dap lys dacB64*	4	Hirota	11

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TABLE 2. Muropeptide composition of the murein synthesized by ether-permeabilized cells of *E. coli* deficient in various PBPs[a]

Muropeptide[b]	Muropeptide content[c] (% of total)							
	KN126 (wild type)	SP1028 (1a⁻)	SP1026 (1b⁻)	SP6 (1a⁻ 2⁻)	PA3092 (wild type)	DL64 (4⁻)	SP5003 (5⁻)	JE5684 (4⁻ 5⁻)
Tri	5.8	5.9	9.6	0.6	8.0	1.7	4.6	1.4
Tetra	16.8	16.0	16.3	17.1	20.2	23.9	11.8	8.2
Penta	1.7	2.0	3.2	2.3	1.2	2.4	5.9	10.4
Tetra-Tetra-UDP	3.2	2.3	2.5	4.3	3.5	2.3	2.0	1.1
Tetra-Penta-UDP	2.9	2.0	—[d]	3.8	2.1	2.5	2.9	4.9
Tetra-Tri	8.8	6.0	17.7	1.8	10.5	5.1	7.5	4.9
Tetra-Tetra	18.0	14.1	14.4	17.6	18.7	20.1	12.2	10.9
Tetra-Anh	1.4	1.1	1.4	1.1	1.5	1.9	1.1	0.5
Tetra-Tetra-Tetra-UDP	2.9	3.0	1.1	4.3	2.9	1.9	2.1	0.9
Tetra-Penta	4.8	4.3	1.3	6.1	1.6	5.5	10.8	16.0
Tetra-Tetra-Penta-UDP	5.5	5.2	0.6	7.6	3.4	4.1	8.8	8.3
Tetra-Tetra-Tri	1.3	1.4	1.0	0.5	1.7	0.8	1.3	0.8
Penta-Anh	0.5	0.4	—	0.3	—	0.2	0.4	0.4
Tetra-Tetra-Tetra	2.6	2.3	2.2	3.3	2.3	2.6	1.9	1.8
Tetra-Tetra-Tetra-Penta-UDP	0.8	0.6	—	1.6	0.2	0.5	1.4	0.8
Tetra-Tetra-Penta	0.6	1.0	—	0.6	—	0.6	2.2	2.3
Tetra-Tri-Anh I	0.8	0.9	0.9	0.7	0.8	0.5	0.9	0.6
Tetra-Tri-Anh II	1.0	1.3	0.7	0.4	1.2	1.5	1.1	1.4
Tetra-Tetra-Tetra-Tetra	0.6	0.3	—	1.0	0.4	0.5	0.4	0.3
Tetra-Tetra-Anh I	1.4	1.2	0.8	2.1	1.6	1.8	1.5	1.0
Tetra-Tetra-Anh II	1.4	1.6	1.0	1.7	2.4	4.6	0.7	2.0
Tetra-Penta-Anh I	0.4	0.4	0.6	0.3	1.2	0.7	2.1	1.4
Tetra-Penta-Anh II	0.6	0.5	0.3	0.5	0.5	0.6	0.3	1.2
Tetra-Tetra-Tetra-Anh	1.2	1.0	0.5	1.5	1.5	1.9	1.2	1.0
Tetra-Tetra-Penta-Anh	—	—	—	0.2	—	0.2	0.4	0.5

[a] Changes of more than 25% from the control are underlined with a solid line, and those with a decrease of more than 25% are indicated with a dashed underline.

[b] Muropeptide structures not relevant for the comparison were omitted from this table. Abbreviations used for the muropeptides were based on their peptide chains; thus, Tri stands for *N*-acetylglucosaminyl (NAcGlc)-*N*-acetylmuramyl (NAcMur)-tripeptide, and Tetra-Penta stands for the cross-linked dimer of NAcGlc-NAcMur-tetrapeptide and NAcGlc-NAcMur-pentapeptide, whereby the tripeptide consists of L-Ala-D-Glu-m-A_2pm, the tetrapeptide of L-Ala-D-Glu-m-A_2pm-D-Ala, and the pentapeptide of L-Ala-D-Glu-m-A_2pm-D-Ala-D-Ala. The peptide chains are cross-linked between m-A_2pm and D-Ala (in position 4). Other abbreviations: Anh, NAcGlc-1,6-anhydro-NAcMur; UDP, UDP-NAcMur.

[c] Murein synthesized as described in the text was digested with muramidase from *Chalaropsis* sp., and the muropeptides were separated by reverse-phase HPLC on a Hypersil ODS-18 column.

[d] —, Not detected.

various PBPs in the complex process of murein synthesis, we analyzed the muropeptide pattern of murein synthesized by mutants defective in PBPs. Murein that was synthesized by ether-permeabilized cells was digested with *Chalaropsis* muramidase, and the released muropeptides were separated by HPLC. The results with mutants with deletions in PBP 1a, 1b, and 5 as well as with point mutations in PBPs 2 and 4 are shown in Table 2. Due to experimental problems with the ether treatment of the filamentous cells of temperature-sensitive PBP 3 mutants, no reliable data for a deficiency in this PBP could be obtained.

No profound changes in the yield of murein synthesis or in the muropeptide pattern (Table 2) could be detected when the bifunctional PBP 1a (SP1028) was deleted. It seems, however, that in ether-permeabilized cells this protein is less active than in vivo, since unlike a deficiency in PBP 1a, a deletion in PBP 1b (SP1026) caused a drastic decrease (about 85%) in overall murein synthesis (Table 3). PBP 1b has been shown already to be the major murein-synthesizing activity in vitro (13, 21), although in vivo PBP 1a can fully compensate for a loss of PBP 1b (20). The analysis of the murein synthesized in vitro by a mutant in PBP 1b (SP1026) showed a number of dramatic differences (Table 2). Whereas tripeptide side chain-carrying monomeric and dimeric muropeptides (tri and tetra-tri) were increased by as much as 100%, we found a drastic reduction in UDP-linked muropeptides, the products of the direct transpeptidation of UDP-muramylpentapeptide, which has recently been described to take place in vitro (8, 10). The latter effect could be due to the reduction of pentapeptide residues in the murein formed, which is known to drive the direct transpeptidation (8). Furthermore, all trimers and tetramers were decreased. This finding is at variance with the results of Tomioka et al. (22), showing that murein formed by PBP 1a in vitro is enriched in oligomeric muropeptides. However, in those experiments the activity of purified PBP 1a was analyzed in an in vitro system consisting of isolated lipid-linked murein precursors and membranes. Ether-permeabilized cells, as used in the present report, more closely represent the in vivo situation. The results, therefore, cannot be compared.

A mutation in PBP 4 (DL64), which shows endopeptidase activity in vitro (7), reduced the amount of tripeptide-containing muropeptides by 50%, or even more in the case of the disaccharide tripeptide (Table 2). The corresponding tetra- and pentapeptide-carrying muropeptides were proportionally increased. This indicates that this PBP very likely

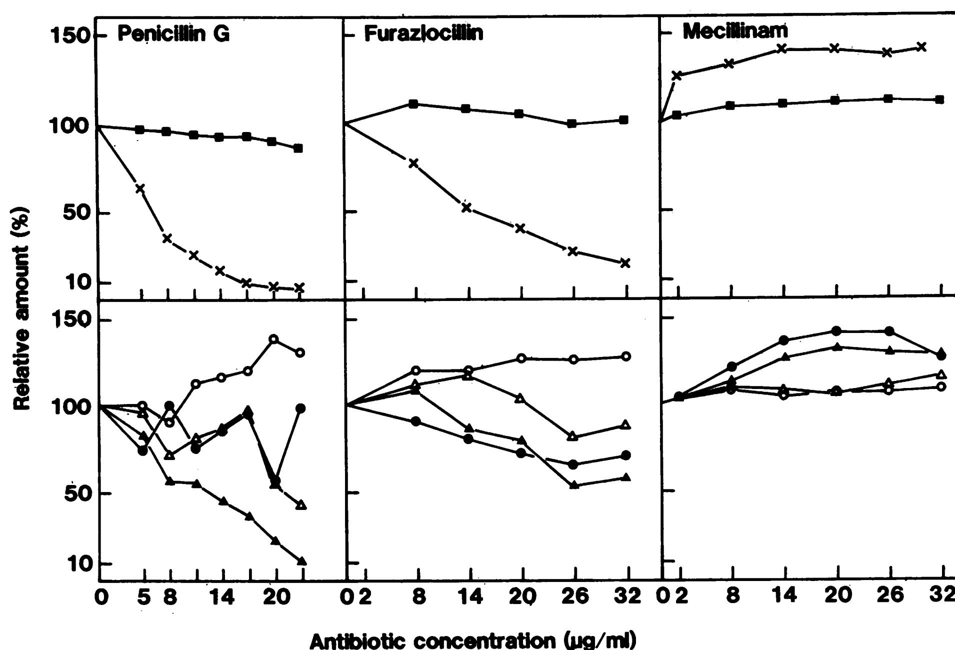

FIG. 1. Effect of penicillin G, furazlocillin, and mecillinam on the formation of different transpeptidation products. Upper panels: In vitro murein synthesis (×) by ether-permeabilized cells of *E. coli* KN126 was followed by incorporation of *N*-acetyl-[^{14}C]glucosamine into SDS-insoluble murein as described in the text. Murein analysis was done by complete digestion with muramidase from *Chalaropsis* sp. and separation of the murein degradation products by reverse-phase HPLC on a Hypersil ODS-18 column. Total cross-linkage of the murein (■) was calculated by the formula given in Table 3, footnote *b*. Lower panels: From the data given in Table 4, the relative amount of different types of cross-linked muropeptides was calculated and plotted versus the concentration of antibiotic present during murein synthesis. Symbols: ○, normal dimers (tetra-tri, tetra-tetra, tetra-penta, tetra-tri-anh I and II, tetra-tetra-anh I and II, tetra-penta-anh I and II); ●, UDP dimers (tetra-tetra-UDP, tetra-penta-UDP); △, normal oligomers (tetra-tetra-tri, tetra-tetra-tetra, tetra-tetra-penta, tetra-tetra-tetra-tetra, tetra-tetra-tetra-anh, tetra-tetra-penta-anh); ▲, UDP oligomers (tetra-tetra-tetra-UDP, tetra-tetra-penta-UDP, tetra-tetra-tetra-penta-UDP) See Table 2, footnote *b*, for abbreviations.

functions as a carboxypeptidase in ether-permeabilized cells. As one would expect, deletion of PBP 5 (SP5003) resulted in an increase in muropeptides with pentapeptide side chains. Most dramatic was the rise of pentapeptidyl-

TABLE 3. Characterization of in vitro murein synthesis by PBP mutants of *E. coli*

Strain (mutation)	Incorporation of label[a] (pmol/mg)	Degree of cross-linkage[b] (%): Total	Degree of cross-linkage[b] (%): UDP-linked muropeptides	Muropeptides with pentapeptidyl residues[c] (% of total)
KN126 (wild type)	191	37.6	10.2	9.7
SP1028 (PBP 1a)	118	37.0	10.0	10.2
SP1026 (PBP 1b)	28	31.0	2.9	5.6
SP6 (PBP 1a and 2)	123	41.4	15.5	13.2
PA3092 (wild type)	166	34.8	7.9	5.6
DL64 (PBP 4)	266	36.0	7.3	9.7
SP5003 (PBP 5)	267	40.2	11.7	20.1
JE5684 (PBP 4 and 5)	270	41.0	11.2	30.1

[a] *N*-Acetyl-[^{14}C]glucosamine incorporation into SDS-insoluble murein by ether-treated cells was determined after incubation for 1 h at 30°C, followed by boiling and filtration of the samples through membrane filters as described in the text.

[b] Cross-linkage of the murein was calculated by the formula: 100 × [(one-half dimer + two-thirds trimer + three-quarters tetramer)/(monomer + dimer + trimer + tetramer)].

[c] The *N*-acetyl-[^{14}C]glucosamine-labeled murein was digested with muramidase from *Chalaropsis* sp., and the muropeptides were separated by reverse-phase HPLC on a Hypersil ODS-18 column.

muropeptides (about 10 times) in a double PBP 4 and 5 mutant (JE5684), as shown in Tables 2 and 3.

A quite interesting finding was an overall increase in UDP-linked muropeptides, the product of the direct transpeptidation in the spherical PBP 2 mutant (SP6). This was clearly seen in a comparison of the degree of normal cross-linkages with UDP-muramylpeptidyl cross-linkages, as summarized in Table 3 for all PBP mutants.

Effect of penicillin, furazlocillin, and mecillinam on in vitro murein synthesis. The amidinopenicillanic acid mecillinam is considered a specific inhibitor of the process responsible for the normal rod shape of *E. coli* (17, 18), whereas furazlocillin is known to specifically inhibit cell division (1). Penicillin G has been shown to affect both processes depending on the concentration used (16). The effect of different concentrations of these antibiotics on in vitro murein synthesis by ether-permeabilized cells was analyzed in detail.

Incorporation of ^{14}C-labeled UDP-*N*-acetylglucosamine into sodium dodecyl sulfate (SDS)-insoluble material was inhibited by penicillin G and furazlocillin but not by mecillinam at the concentrations used (Fig. 1, upper panels). The muropeptide pattern of the murein synthesized in vitro in the presence of these β-lactams is presented in Table 4. The most striking changes in the muropeptide composition were an increase in tri monomers and tetra-tri dimers of about 100% in the presence of penicillin G and the dramatic accumulation of pentapeptide side chains at the expense of tetrapeptidyl muropeptides in the presence of furazlocillin. The penicillin G effect found its expression at the highest concentration used (23 μg/ml), whereas the furazlocillin-

TABLE 4. Muropeptide analysis of *E. coli* KN126 murein synthesized by ether-treated cells in the presence of β-lactam antibiotics[a]

Muropeptide	Muropeptide content[b] (% of total)																				
	Penicillin G (μg/ml)								Furazlocillin (μg/ml)						Mecillinam (μg/ml)						
	0	5	8	11	14	17	20	23	0	8	14	20	26	32	0	2	8	14	20	26	32
Tri	5.3	8.2	7.3	9.5	9.1	9.4	8.4	9.3	5.3	3.4	3.5	3.4	3.1	3.0	5.3	5.8	5.6	5.2	5.1	5.3	5.0
Tetra	18.1	15.2	11.0	14.6	16.8	17.7	20.6	20.9	18.1	4.4	3.6	3.4	3.7	3.0	18.1	14.6	12.1	11.8	11.8	10.8	11.1
Penta	1.7	1.9	2.1	2.8	3.1	3.6	4.0	4.2	1.7	10.6	12.9	16.6	18.7	18.6	1.7	1.9	2.1	2.2	2.2	2.4	2.5
Tetra-Tetra-UDP	3.0	2.2	3.5	2.8	3.0	3.5	2.1	4.1	3.0	0.8	0.7	0.2	0.5	0.5	3.0	3.3	3.5	3.6	3.7	3.6	3.3
Tetra-Penta-UDP	3.1	2.3	2.6	1.8	2.2	2.3	1.4	1.9	3.1	4.7	4.2	4.2	3.5	3.8	3.1	3.1	3.9	4.7	4.9	5.0	4.4
Tetra-Tri	8.8	8.5	9.0	12.5	13.1	13.9	17.4	19.0	8.8	8.5	7.1	6.8	6.5	6.0	8.8	9.4	9.5	8.8	8.6	8.8	8.3
Tetra-Tetra	18.0	14.4	12.4	14.9	15.7	17.1	18.0	16.4	18.0	6.3	5.4	4.4	4.5	3.4	18.0	18.0	18.4	17.4	17.6	17.1	17.6
Tetra-Anh	1.4	0.5	1.1	1.0	0.9	—[c]	—	—	1.4	0.4	0.3	—	—	—	1.4	1.2	1.1	1.2	1.1	1.2	1.3
Tetra-Tetra-Tetra-UDP	3.1	2.2	1.8	2.1	1.7	1.5	0.9	1.0	3.1	0.7	0.6	—	—	—	3.1	3.1	2.5	3.1	2.4	2.3	2.4
Tetra-Penta	4.6	4.8	3.5	3.9	4.5	2.7	4.5	3.3	4.6	20.7	22.6	26.6	26.4	28.1	4.6	4.7	5.7	5.3	5.9	6.4	6.8
Tetra-Tetra-Penta-UDP	5.3	4.6	2.7	2.6	2.5	1.9	1.2	—	5.3	8.1	6.5	6.4	4.4	4.7	5.3	5.7	6.9	7.5	8.0	8.4	8.0
Tetra-Tetra-Tri	1.2	0.7	0.7	1.2	1.5	1.7	—	—	1.2	0.6	0.7	0.7	0.5	0.5	1.2	1.4	1.4	1.3	1.2	1.2	1.2
Penta-Anh	0.3	0.6	0.4	0.4	0.3	—	—	—	0.3	0.7	0.7	—	0.4	—	0.3	0.3	0.3	0.3	0.2	0.3	0.3
Tetra-Tetra-Tetra	2.6	2.6	2.0	1.9	2.1	2.9	1.1	1.0	2.6	1.4	1.9	1.1	0.9	0.7	2.6	2.5	2.6	2.6	2.5	2.8	2.9
Tetra-Tetra-Tetra-Penta-UDP	0.8	0.8	0.7	0.4	—	—	—	—	0.8	1.2	0.8	0.9	0.5	0.6	0.8	0.8	1.0	1.0	0.9	1.2	1.3
Tetra-Tetra-Penta	0.5	0.5	0.8	0.5	—	—	—	—	0.5	3.0	2.9	3.2	2.3	2.6	0.5	0.8	0.9	0.9	0.9	1.0	1.1
Tetra-Tri-Anh I	0.8	0.6	0.9	1.1	1.0	1.7	0.8	2.7	0.8	0.9	0.7	0.6	0.4	0.6	0.8	0.8	0.8	0.7	0.6	0.6	0.7
Tetra-Tri-Anh II	1.0	2.4	2.0	2.4	2.3	2.9	2.8	2.6	1.0	2.1	2.3	1.8	1.9	1.3	1.0	1.3	1.7	1.9	2.1	2.0	2.0
Tetra-Tetra-Tetra-Tetra	0.7	0.7	—	0.3	—	—	—	—	0.7	0.2	0.4	0.4	0.3	0.7	0.7	0.6	0.6	0.7	0.7	0.7	0.6
Tetra-Tetra-Anh I	1.4	2.6	2.5	3.1	2.8	3.5	3.6	2.8	1.4	1.0	1.0	0.9	0.5	0.7	1.4	1.8	1.8	1.6	1.5	1.6	1.5
Tetra-Tetra-Anh II	1.9	2.6	2.4	2.5	2.7	2.2	3.0	2.0	1.9	1.3	1.2	1.2	1.0	1.0	1.9	1.8	1.8	2.4	2.6	2.6	2.4
Tetra-Penta-Anh I	0.6	0.9	0.9	1.1	1.0	0.6	1.0	—	0.6	1.8	2.1	2.3	2.6	3.0	0.6	0.7	0.7	0.7	0.8	0.8	0.9
Tetra-Penta-Anh II	0.5	0.7	0.4	0.6	0.5	0.4	0.7	—	0.5	2.2	2.4	2.8	3.4	3.6	0.5	0.6	0.5	0.6	0.5	0.6	0.7
Tetra-Tetra-Tetra-Anh	1.3	1.5	1.0	1.2	1.9	1.4	2.6	1.7	1.3	0.8	0.6	—	—	—	1.3	1.2	1.4	1.3	1.4	1.3	1.5
Tetra-Tetra-Penta-Anh	—	—	—	—	—	—	—	—	—	1.0	0.9	1.1	1.1	1.1	—	—	—	—	—	—	—

[a] See Table 2, footnotes *a* and *b*.
[b] Murein synthesized in the presence of the indicated amounts of antibiotics as described in the text was digested with muramidase from *Chalaropsis* sp., and the muropeptides were separated by reverse-phase HPLC on a Hypersil ODS-18 column.
[c] —, Not detected.

induced alteration in murein structure was evident at the lowest concentration used. It seems that furazlocillin inhibits the DD-carboxypeptidase more effectively than penicillin G, which does not necessarily conflict with the well known fact that furazlocillin preferentially binds to PBP 3 (1). Most interesting are the differences in the amount of UDP-muramyl-linked muropeptides as well as trimers and tetramers, as shown in the next paragraph.

***β*-Lactam sensitivity of the formation of the different transpeptidation reaction products.** Recent studies on murein synthesis in vitro indicated the presence of a specific transpeptidation reaction. Direct transpeptidation bypassing the formation of a lipid intermediate preferentially yields UDP-muramylpeptide-linked trimers and tetramers with the UDP-muramylpeptide in the acceptor site (8). This reaction therefore seems to be catalyzed by a system normally responsible for the formation of oligomeric compounds. Interestingly, from the muropeptide analysis data (Table 4), it can be seen that the formation of UDP-muramyl-linked muropeptides as well as the formation of trimers and tetramers differed in their sensitivity to β-lactam antibiotics; this is summarized in Fig. 1.

Although the overall degree of cross-linkage of the newly synthesized murein remained constant in the presence of a wide range of concentrations of penicillin G, mecillinam, and furazlocillin (Fig. 1, upper panels), the formation of UDP-linked muropeptides and oligomers (trimers and tetramers) was severely decreased by penicillin G and to a lesser degree by furazlocillin; in the presence of mecillinam the relative amount of these muropeptides was slightly increased (Fig. 1, lower panels). The formation of normal dimers clearly displayed a different sensitivity to penicillin G, furazlocillin, and mecillinam compared with the formation of both UDP-linked muropeptides (dimers and oligomers) and normal oligomers (trimers and tetramers).

DISCUSSION

The results presented here reveal that the formation of hyper-cross-linked muropeptides, i.e., trimers and tetramers, is highly sensitive to penicillin G and furazlocillin, whereas the synthesis of normal dimeric muropeptides is relatively resistant to these β-lactams. This indicates that the reaction mechanism or more likely the transpeptidases involved differ for the formation of dimers and oligomers.

Septation of *E. coli* is known to be specifically inhibited by furazlocillin and low concentrations of penicillin G, whereas cell elongation continues undisturbed under these conditions (1, 16). One could therefore speculate that inhibition of cell division by these antibiotics results from a lack in the synthesis of hyper-cross-linked muropeptides. On this ground, we would like to propose that two distinct transpeptidation systems exist in *E. coli*. One enzyme that specifically catalyzes the synthesis of trimers and tetramers

may be responsible for crosswall formation; a second transpeptidase that forms dimers may be mainly involved in longitudinal wall growth.

Mecillinam-treated cells and PBP 2-deficient mutants do not elongate but grow as spheres (14, 17). It seems reasonable to assume that in these spherical cells murein is synthesized by the enzyme system normally involved in the formation of septum material but without division taking place. Consistent with our proposal of two specific transpeptidases, such cells were indeed found to synthesize increased amounts of trimers and tetramers as well as UDP-muramyl-linked muropeptides in vitro.

The recently discovered direct transpeptidation reaction that takes place under in vitro conditions (8, 10), yielding UDP-muramyl-linked muropeptides, is not only effectivly inhibited by penicillin G and furazlocillin, but in addition has previously been shown to preferentially produce trimers (8). It seems, therefore, that the direct transpeptidation reaction is catalyzed by the enzyme normally involved in trimer and tetramer formation. Interestingly, the features of the direct transpeptidation reaction strongly resemble the reverse transpeptidase reaction, as recently demonstrated for PBP 3 (15), the specific target of furazlocillin. In both reactions the donor of the transpeptidation is the insoluble newly synthesized and therefore pentapeptide-rich murein and the acceptor a soluble precursor molecule, an UDP-*N*-acetylmuramylpeptide. This is in contrast to normal transpeptidation, in which the high-molecular-weight murein functions as an acceptor and the donor is the nascent murein or soluble murein precursor. The enzyme catalyzing the formation of oligomers as well as the in vitro direct transpeptidation reaction may indeed be PBP 3, which has been reported to be a transpeptidase specifically involved in cell division (1).

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