Peptidoglycan biosynthesis in *Escherichia coli*: variations in the metabolism of alanine and D-alanyl-D-alanine

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The *in vivo* functioning of the alanine/D-alanyl-D-alanine pathway of *Escherichia coli* was investigated by determining precursor pool levels and specific enzyme activities under various growth conditions. Cells grown on D- or L-alanine showed several remarkable features compared with cells grown on other carbon sources: 10-fold higher values of the D-alanyl-D-alanine and the UDP-MurNAc-pentapeptide pools, a 240-fold increase of the alanine racemase activity, and the absence of bacteriolysis after treatment with D-cycloserine at high concentrations ($50 \mu\text{g ml}^{-1}$). In cells grown on glucose, D-cycloserine ($1 \mu\text{g ml}^{-1}$) led to depletion of the D-alanyl-D-alanine pool and to lysis, which was efficiently antagonized by chloramphenicol. A threefold increase of the dipeptide pool was observed when cells were treated with chloramphenicol alone. The alanine racemase activity was lowest in glucose-grown cells and the D-alanine:D-alanine ligase and D-alanyl-D-alanine-adding activities were the same whatever the carbon source. Molecular masses of 53–56 kDa and 56–60 kDa were estimated for the partially purified inducible alanine racemase and D-alanine:D-alanine ligase respectively.

Introduction

In the biosynthesis of bacterial peptidoglycan the formation of D-alanyl-D-alanine from L-alanine via D-alanine appears as a side pathway linked to the main reaction sequence at two points: the addition of L-alanine to UDP-*N*-acetylmuramic acid and the addition of D-alanyl-D-alanine to the UDP-MurNAc-tripeptide nucleotide (Rogers *et al.*, 1980). A racemase activity converts L-alanine into D-alanine and a ligase activity catalyses the formation of D-alanyl-D-alanine from D-alanine (Rogers *et al.*, 1980). In *Escherichia coli* this pathway has already been investigated to some extent (Lambert & Neuhaus, 1972*a*; Lugtenberg, 1972; Ishiguro & Ramey, 1978; Lugtenberg & van Schijndel-van Dam, 1973; Wild *et al.*, 1985). The alanine racemase activity in *E. coli* is due to two distinct gene products (Wild *et al.*, 1985). The predominant racemase, coded by the *dadX* gene, is inducible by alanine and repressible by glucose (Wild *et al.*, 1985). Homogeneous preparations of this activity were obtained from two strains of *E. coli* (Lambert & Neuhaus, 1972*b*; Wang & Walsh, 1978). The other racemase, which is probably the product of the *alr* gene, is constitutive in respect of both alanine and

glucose (Wijsman, 1972; Wild *et al.*, 1985). More recently it has been established that there are two distinct genes (*ddlA* and *ddlB*) encoding D-alanine:D-alanine ligases (Robinson *et al.*, 1986; Zawadzke *et al.*, 1991). Both DdlA and DdlB enzymes have been overproduced and purified. They possess very similar kinetic characteristics.

The purpose of the present work was to reach a better understanding of the *in vivo* functioning of the alanine/D-alanyl-D-alanine pathway by determining the values of simple parameters such as peptidoglycan precursor pool levels and specific activities of the racemase and ligase activities under different conditions of growth or after treatment with antibiotics. Recently, a similar approach has led to a number of important conclusions concerning the uridine nucleotide pathway of peptidoglycan biosynthesis (Mengin-Lecreulx *et al.*, 1982, 1983, 1989; Mengin-Lecreulx & van Heijenoort, 1985, 1990).

Methods

Bacterial strain, growth conditions and crude enzyme preparations. *E. coli* K12 HfrH (*thi relA*), used throughout this work, was grown at 37 °C as previously described (Mengin-Lecreulx *et al.*, 1982) either in LB medium or in minimal medium M63 supplemented with thiamin (0.5 mg l^{-1}) and 0.2% of one of the various carbon sources listed in

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Tables 1 and 2. In all cases, 2 litre flasks containing 500 ml medium were inoculated with 5 ml of overnight precultures in the same medium and growth was monitored at 600 nm in a Gilford spectrophotometer model 240. Cell counts and dry cell weights were determined as described earlier (Mengin-Lecreux & van Heijenoort, 1985). Crude enzyme preparations were obtained as described previously (Mengin-Lecreux *et al.*, 1982).

Chemicals and analytical procedures. D-Alanyl-D-alanine and D-[^{14}C]alanyl-D-[^{14}C]alanine (2.2 GBq mmol $^{-1}$) were prepared as previously described (Gondré *et al.*, 1973). D- and L-amino acid oxidases and L-glutamate:pyruvate transaminase were purchased from Serva. Protein was estimated by the Lowry method, with bovine serum albumin as a standard. Peptidoglycan nucleotide precursors were isolated and quantified as previously described (Mengin-Lecreux *et al.*, 1982, 1983).

Content of alanine and D-alanyl-D-alanine in cells. Cells from 1 or 2 litres of cultures were rapidly chilled to 0 °C and harvested in the cold. The alanine content of extracts obtained by treating cells with boiling water and cold 5% (w/v) trichloroacetic acid was measured with a Biotronik model LC 2000 automatic acid analyser. The relative abundances of D- and L-alanine were estimated by the procedure of Wellner & Lichtenberg (1971), involving specific degradation of either isomer by a D- or L-amino acid oxidase, or that of the L-isomer by a L-glutamate:pyruvate transaminase. In all cases, amounts were estimated from the differences in alanine contents before and after enzyme treatment.

D-Alanyl-D-alanine was isolated from cell extracts by a three-step procedure involving: (i) gel filtration of the bacterial extract on fine Sephadex G-25 (Mengin-Lecreux *et al.*, 1982), (ii) chromatography on Whatman 3MM filter paper in the solvent system 1-butanol/acetic acid/water (4:1:1, by vol.) for 24 h, and (iii) rheophoresis on the same paper in 0.1 M-formic acid (pH 2.2) for 2 h at 20 V cm $^{-1}$. To ensure its detection and the evaluation of its recovery at each step, a given amount (2000 Bq) of D-[^{14}C]alanine-D-[^{14}C]alanine was initially added to the extracts. Samples of isolated D-alanyl-D-alanine were quantified with the Biotronik analyser under conditions allowing its complete separation from eventual traces of amino acids. The D-alanine content of the isolated dipeptide was determined after acid hydrolysis.

Assays for alanine racemase and D-alanine:D-alanine ligase. The enzymes were assayed in crude extracts of *E. coli* K12 HfrH prepared as previously described (Mengin-Lecreux *et al.*, 1982). The time range of incubation and the amount of extract yielding linear kinetics were established and used in each case.

Alanine racemase was determined in either direction (L-alanine $\rightarrow$ D-alanine or D-alanine $\rightarrow$ L-alanine). Reaction mixtures, containing 50 mM-sodium phosphate buffer, pH 8.0, various amounts of L- or D-[^{14}C]alanine (2000 Bq) and enzyme (3 μg protein) in a total volume of 50 μl were incubated for 15 min at 37 °C. After stopping the reaction by heating at 100 °C for 5 min, a procedure similar to that described by Lambert & Neuhaus (1972a) was used for the enzymic deamination of either L- or D-alanine to pyruvate. Finally, [^{14}C]alanine was separated from [^{14}C]pyruvate by rheophoresis on Whatman 3MM filter paper in 0.1 M-formic acid (pH 2.2) for 2 h at 20 V cm $^{-1}$, located by overnight autoradiography (3 M, Medical X-ray film), recovered and its radioactivity measured.

D-Alanine:D-alanine ligase was assayed by the method of Lugtenberg *et al.* (1972, 1973), by following the formation of radioactive UDP-MurNAc-pentapeptide from D-[^{14}C]alanine. Some minor modifications were introduced. After 30 min incubation at 37 °C, the reaction was stopped by addition of 10 μl glacial acetic acid and the same electrophoretic conditions as those of the racemase assay were used to separate radioactive alanine, D-alanyl-D-alanine and UDP-MurNAc-pentapeptide.

The activity of the D-alanyl-D-alanine-adding enzyme was assayed as previously described (Mengin-Lecreux *et al.*, 1982).

Partial purification and molecular mass of the alanine racemase and D-alanine:D-alanine ligase. The enzymes were partially purified from cells grown in rich medium, or in minimal medium with glucose or L-alanine. Crude cell extracts from 1 litre cultures were chromatographed on a MonoQ anion-exchange column (50 $\times$ 5 mm i.d.) monitored by an FPLC apparatus (Pharmacia) equipped with a detector set at 274 nm. Elutions were carried out at a flow rate of 1 ml min $^{-1}$: 10 min with 0.01 M-Tris/0.005 M-phosphate buffer, pH 7; 80 min with a linear gradient from 0 to 0.6 M NaCl in the same buffer. This technique efficiently separates the proteins of the *E. coli* crude extract into 30 peaks. The racemase activity was recovered in a peak eluted at 28 min with a 73% yield and an 11-fold purification. The ligase activity was recovered in a peak eluted at 38 min with a 25% yield and a 6-fold purification. The apparent molecular masses of the alanine racemase and D-alanine:D-alanine ligase were determined by gel filtration of active fractions (concentrated on Amicon PM10 filters) on a Superose 12 column (300 $\times$ 5 mm i.d., Pharmacia) monitored with the same apparatus as above. The column was calibrated with alcohol dehydrogenase, bovine serum albumin, ovalbumin, chymotrypsinogen, myoglobin and cytochrome c.

Results

Variations of the pool levels of alanine, D-alanyl-D-alanine and peptidoglycan nucleotide precursors

In exponential-phase cells of *E. coli* K12 the D- and L-alanine pools were higher in cells grown in rich medium than in cells grown in minimal medium (Table 1). In all cases the L-isomer accounted for 60–75% of the alanine quantified. It is noteworthy that the alanine pools were not higher in L-alanine-grown cells than in glucose-grown cells and that they were particularly low in pyruvate-grown cells. The assay developed for the determination of the D-alanyl-D-alanine cell pool gave reproducible results and it was verified that the dipeptide recovered contained only D-alanine. The dipeptide pool was far lower than the alanine pool and did not vary greatly with the growth medium, except in alanine-grown cells, where an 8- to 10-fold increase was observed (Table 1). The D-alanyl-D-alanine pool decreased significantly only in stationary-phase cells grown in minimal medium (Table 1).

When considering the variations of the pool levels of the peptidoglycan nucleotide precursors with various carbon sources (Table 1), the most striking feature was the increase in UDP-MurNAc-pentapeptide in exponential-phase cells grown on D- or L-alanine or on pyruvate. As previously described (Mengin-Lecreux & van Heijenoort, 1985) no major variations were observed with stationary-phase cells grown in rich medium. However, in stationary-phase cells grown on glucose or alanine the decrease of the UDP-GlcNAc, D-alanyl-D-alanine and UDP-MurNAc-pentapeptide pools was

Table 1. Pool levels of peptidoglycan precursors in *E. coli* K12 cells as a function of growth medium and growth phase

Growth medium (generation time)	Pool levels [nmol (g dry cell weight of bacteria) ⁻¹]								
	D-Ala	L-Ala	D-Ala- D-Ala	UDP- GlcNAc	UDP- MurNAc	UDP-MurNAc L-alanine	UDP-MurNAc dipeptide	UDP-MurNAc tripeptide	UDP-MurNAc pentapeptide
Exponential phase*									
LB (36 min)	3200	10100	150	600	73	22	18	9	626
M63 + glucose (55 min)	2100	5700	230	850	100	5	5	20	250
M63 + pyruvate (87 min)	500	900	280	915	112	6	5	45	1228
M63 + D-Ala (97 min)	1360	1815	1650	650	98	4	5	31	1518
M63 + L-Ala (110 min)	2300	3500	2200	800	80	15	2	20	1700
Stationary phase†									
LB	320	640	170	390	25	2	100	35	615
M63 + glucose	50	160	30	13	435	49	135	4	52
M63 + L-Ala	240	470	40	11	670	4	200	11	39

* In all cases cells were harvested at an OD₆₀₀ of 0.7, which corresponded to the mid-exponential phase and to 180 mg bacterial dry weight per litre of culture.

† Overnight stationary phase cells.

Table 2. Specific activities of the enzymes involved in the metabolism of alanine and D-alanyl-D-alanine in *E. coli* K12 as a function of growth medium and growth phase

Growth medium	Generation time (min)	Bacterial dry wt [mg (l culture) ⁻¹]*	Specific activities [nmol min ⁻¹ (mg protein) ⁻¹]		
			Alanine racemase	D-Ala :D-Ala ligase	D-Ala-D-Ala adding enzyme
LB	36	100	8	0.120	2.0
	36	180	11	0.100	2.0
	36	235	11	0.100	1.8
	ND	470†	10	0.095	1.8
	ND	645†	10	0.085	1.7
	ND	880‡	7	0.070	1.6
M63 + glucose	55	180	0.7	0.100	2.0
M63 + glycerol	70	180	2.9	0.070	1.9
M63 + pyruvate	87	180	12.6	ND	2.3
M63 + D-alanine	97	180	116	ND	ND
M63 + L-alanine	110	180	170	0.080	1.9
M63 + glucosamine	100	180	2.4	0.085	1.8
M63 + rhamnose	190	180	2.8	0.065	2.0

ND, Not determined.

* Unless otherwise indicated, cells were harvested at different times of the exponential phase.

† Early stationary phase cells.

‡ Overnight stationary phase cells.

accompanied by a sharp increase of the UDP-MurNAc and UDP-MurNAc-dipeptide pools.

Effects of growth conditions on the enzymes of the alanine/D-alanyl-D-alanine pathway

The specific activities of alanine racemase, D-alanine:D-alanine ligase and D-alanyl-D-alanine-adding enzyme were compared under various growth conditions (Table 2). In cells grown on minimal medium containing a carbon source other than alanine or pyruvate, fairly

constant values were encountered whatever the growth rate. In cells grown on rich medium the ligase and the D-alanyl-D-alanine-adding activities were the same as in cells grown in minimal medium, but the racemase activity was severalfold higher. In late-stationary-phase cells high levels of all three activities remained; the greatest variation observed was a 40% decrease in ligase activity. As previously described (Wijsman, 1972; Lambert & Neuhaus, 1972a; Wild *et al.*, 1985), growth on D- or L-alanine as carbon source led to a great increase in alanine racemase activity (Table 2). With the K12

Table 3. Effects of D-cycloserine and chloramphenicol on the pool levels of the peptidoglycan precursors of *E. coli* K12

Each antibiotic was added at the indicated final concentration to exponential-phase cells (130 mg bacterial dry weight l^{-1}) growing in glucose minimal medium and 20 or 30 min thereafter peptidoglycan nucleotide precursors were extracted and quantified.

Treatment	Final concn (μg ml ⁻¹)	Time before harvest (min)	Pool levels [nmol (g dry cell weight of bacteria) ⁻¹]					
			D-Ala-D-Ala	UDP-GlcNAc	UDP-MurNAc	UDP-MurNAc dipeptide	UDP-MurNAc tripeptide	UDP-MurNAc pentapeptide
Control		—	155	990	185	6	43	200
D-Cycloserine	1*	20	ND	972	189	101	2090	50
D-Cycloserine	1	30	<2	860	174	130	2380	30
Chloramphenicol	200	30	490	5650	515	30	12	3050
D-Cycloserine + chloramphenicol	1	30						
	200†	10	350	2490	240	305	1570	620
D-Cycloserine + chloramphenicol	1	30						
	200	30	460	4650	500	30	12	3000

ND, Not determined.

* This concentration was chosen so as to promote cell lysis just 30 min after addition of D-cycloserine.

† In this case chloramphenicol was added 20 min after D-cycloserine and cells were harvested 10 min later.

strain used a 240-fold ratio was observed between the highest racemase activity (growth on L-alanine) and the lowest (growth on glucose). With late-stationary-phase cells grown on alanine a high level of racemase activity and a normal level of ligase activity were still present (data not shown). A molecular mass of 53–56 kDa was estimated for the inducible racemase from cells grown on L-alanine and should be compared to the values of 98 kDa and 103 kDa determined in the case of *E. coli* strains W and B respectively (Lambert & Neuhaus, 1972a; Wang & Walsh, 1978). The enzyme from *E. coli* B is a dimer composed of identical subunits each carrying one active site and one pyridoxal phosphate molecule (Wang & Walsh, 1978). The molecular mass of the partially purified ligase activity from cells grown in minimal or rich medium was estimated as 56–60 kDa. As previously observed (Lugtenberg, 1972), D-alanyl-D-alanine strongly inhibited the ligase activity tested *in vitro*: 13% inhibition at 10^{-6} M , 30% at 10^{-5} M , 77% at 10^{-4} M and 94% at 10^{-3} M .

Effects of D-cycloserine and chloramphenicol

In vitro, D-cycloserine is an effective inhibitor of both the L-alanine racemase and D-alanine:D-alanine ligase activities of *E. coli* (Lambert & Neuhaus, 1972b; Lugtenberg, 1972; Lugtenberg & van Schijndel-van Dam, 1973; Wang & Walsh, 1978). As expected, the treatment of exponential-phase cells with D-cycloserine led to a strong depletion of the D-alanyl-D-alanine pool (Table 3). Normally, D-alanyl-D-alanine is added to UDP-MurNAc-tripeptide to yield UDP-MurNAc

pentapeptide (Duncan *et al.*, 1990; Rogers *et al.*, 1980). Therefore its depletion led to a sharp decrease of the UDP-MurNAc-pentapeptide pool, and concomitantly to a considerable increase of the UDP-MurNAc-tripeptide pool and also to some extent to that of the UDP-MurNAc-dipeptide (Table 3). Such variations of the UDP-MurNAc-tripeptide and UDP-MurNAc-pentapeptide pools upon D-cycloserine treatment have been previously reported (Ishiguro & Ramey, 1978; Lugtenberg *et al.*, 1972; Neuhaus *et al.*, 1972). The consequence of these variations was a slow-down or arrest of peptidoglycan synthesis and for appropriate concentrations of D-cycloserine a commitment to cell lysis (Fig. 1a). This was in agreement with the general idea that the inhibition of any step of peptidoglycan synthesis in exponential-phase cells of *E. coli* will lead to autolysis (Leduc *et al.*, 1982). However, no cell lysis was observed when exponential-phase cells grown on L-alanine were treated with D-cycloserine, even at a high concentration of $50 \mu\text{g ml}^{-1}$ (Fig. 1b). This effect was specific for D-cycloserine since antibiotics, such as fosfomycin and cephaloridine, affecting other steps in peptidoglycan biosynthesis, were bacteriolytic in the same way as with cells of similar generation time growing on D-glucosamine (Fig. 1b). Presumably, other inhibitors of the D-alanine branch might also have the same effect as D-cycloserine.

The inhibition of D-cycloserine-induced lysis was further examined by blocking protein synthesis with chloramphenicol. As previously described (Brock, 1961; Mengin-Lecreulx *et al.*, 1989) addition of chloramphenicol alone at $200 \mu\text{g ml}^{-1}$ to growing *E. coli* cells had

Fig. 1. Effect of D-cycloserine, cephaloridine and fosfomycin on growing cells of *E. coli* K12 HfrH. Cells were grown exponentially in minimal medium with D-glucosamine (a) or L-alanine (b) as sole carbon source. Different cell treatments were performed when the OD_{600} of the cultures reached 0.5 (arrow, approx. 130 mg dry cell weight per litre of culture). ●, Control without treatment; ■, D-cycloserine ($50 \mu\text{g ml}^{-1}$); ○, fosfomycin ($200 \mu\text{g ml}^{-1}$); □, cephaloridine ($10 \mu\text{g ml}^{-1}$).

an immediate bacteriostatic effect. When it was added together with D-cycloserine the same bacteriostatic effect was observed, but no cell lysis was detectable even after 2 h (Fig. 2). In this case D-cycloserine was used at a concentration ($1 \mu\text{g ml}^{-1}$) that normally promotes cell lysis after 30 min. To exclude possible interactions between the drugs, chloramphenicol was added 20 min after D-cycloserine; again no lysis was observed (Fig. 2). It had been previously established that chloramphenicol alone leads to considerable increases of the UDP-GlcNAc and UDP-MurNAc-pentapeptide pools (Mengin-Lecreux *et al.*, 1989). The present study showed that this was also true for the D-alanyl-D-alanine pool (Table 3). These increases still occurred when chloramphenicol was added together with D-cycloserine or even 20 min after (Table 3).

Fig. 2. Effect of chloramphenicol on D-cycloserine-induced cell lysis. Cells were grown exponentially in minimal medium supplemented with glucose. Different cell treatments were performed when the OD_{600} of the cultures reached 0.5 (arrow, approx. 130 mg dry cell weight per litre of culture). ●, Control without treatment; ■, D-cycloserine ($1 \mu\text{g ml}^{-1}$); □, chloramphenicol ($200 \mu\text{g ml}^{-1}$) or chloramphenicol ($200 \mu\text{g ml}^{-1}$) plus D-cycloserine ($1 \mu\text{g ml}^{-1}$) [results identical]; ○, D-cycloserine ($1 \mu\text{g ml}^{-1}$) for 20 min and then chloramphenicol ($200 \mu\text{g ml}^{-1}$).

Discussion

The increase in alanine racemase activity observed with cells grown on alanine, as compared to those grown on glucose, was in agreement with previous results (Lambert & Neuhaus, 1972a; Wild *et al.*, 1985), but the increase was higher with the K12 strain used here. From the study of the properties of mutants it was previously inferred that L-alanine could be the inducer of the *dad* operon containing the *dadX* gene for the inducible racemase (Wild *et al.*, 1985). This implies a certain increase of the cell alanine pool upon growth on alanine. However, our results show that the D- and L-alanine pools of cells growing on alanine are the same as or lower than those of cells grown on glucose. A similar result led Lambert & Neuhaus (1972a) to favour derepression as the possible mechanism that gives rise to higher levels of racemase activity. A possible explanation is that these pools are grossly underestimated owing to rapid variations during the cooling, harvesting and washing of cells prior to extraction by boiling. Possibly, the depletion of the alanine pools could be due to a quicker arrest of uptake than consumption. It should be emphasized that in exponential-phase cells growing on alanine the

turnover of a 5×10^{-3} M alanine pool, which is sufficient to promote a high level of racemase activity (Lambert & Neuhaus, 1972a), can be estimated at a few seconds. Finally, it should be stressed that the data of Wild *et al.* (1985) on the induction of the *dad* operon do not entirely exclude the possibility of an inducer molecule other than alanine.

The 10-fold higher D-alanyl-D-alanine pool of cells growing on alanine, as compared to that observed with other carbon sources, was a remarkable feature, which could perhaps be the consequence of a putative high alanine pool. This dipeptide pool was apparently not depleted during the isolation procedures. This suggests that once synthesized, in contrast to alanine, D-alanyl-D-alanine is not extensively used for metabolic purposes other than peptidoglycan synthesis by its addition to UDP-MurNAc-tripeptide. It can be estimated that the 10^{-3} M dipeptide pool of cells grown on alanine can sustain peptidoglycan synthesis for at least a quarter of a generation. It was also noteworthy that a threefold increase of the D-alanyl-D-alanine pool was observed after addition of chloramphenicol. This could perhaps be accounted for by the 100% increase of the alanine pool observed after inhibition of protein synthesis by chloramphenicol or valine (data not shown). Another interesting feature of cells growing on alanine or pyruvate was their high UDP-MurNAc-pentapeptide pool. Since no significant increase of the D-alanyl-D-alanine pool was observed with cells growing on pyruvate, it is presently difficult to assess whether or not there is a direct correlation between the pool levels of these two precursors.

Both DdlA and DdlB D-alanine:D-alanine ligases are inhibited to the same extent by their reaction product D-alanyl-D-alanine (Zawadzke *et al.*, 1991). This corresponds to the inhibitions observed previously with crude extracts (Lugtenberg, 1972) or here with the partially purified activity. If this inhibitory effect has a meaning *in vivo*, over 90% of the ligase activity would be inhibited in cells growing on alanine, in which the D-alanyl-D-alanine pool reaches 10^{-3} M. Not only is the ligase activity more or less constant under the different growth conditions, but it is also in great excess in the cell. As previously stated (Park, 1987), the tight regulation by D-alanyl-D-alanine thus appears to be necessary, since without it the entire pool of L-alanine would be converted to D-alanyl-D-alanine. The question remains as to why there are two genes for two distinct ligases with similar properties (Zawadzke *et al.*, 1991). It has not yet been established whether only one or both are expressed in normal growing cells. The native molecular masses of DdlA and DdlB have been estimated at 58–62 kDa and 63–65 kDa respectively (Zawadzke *et al.*, 1991). The value of 56–60 kDa determined here for the ligase

activity could correspond to DdlA. But this is insufficient to assess whether DdlA is predominant in normally growing cells and DdlB is not expressed.

With the absence of D-cycloserine-induced lysis of cells grown on alanine and its inhibition by chloramphenicol in cells grown on glucose, the data reported here afford two interesting examples of the as yet poorly understood correlations between peptidoglycan synthesis, protein synthesis and autolysis. In cells grown on L-alanine the induced racemase converts L-alanine into D-alanine (Lambert & Neuhaus, 1972a), which in turn is converted into pyruvate by the induced D-alanine dehydrogenase (Franklin & Venables, 1976; Wild & Obrepalska, 1982; Wild *et al.*, 1985). Therefore, the inhibition of racemase activity by D-cycloserine affects not only peptidoglycan metabolism but also the entire cell metabolism, and in particular protein synthesis. In many circumstances, autolysis of *E. coli* cells is known to be dependent on active protein synthesis (Höltje & Tuomanen, 1991; Leduc *et al.*, 1982). The lytic effect of D-cycloserine on glucose-grown cells correlated well with the observed variations of the D-alanyl-D-alanine and UDP-MurNAc-peptide pools, leading to arrest of peptidoglycan synthesis. The effect of chloramphenicol on the D-cycloserine-induced lysis of these cells remarkably paralleled the restoration of these precursor pools. This behaviour was reminiscent of the antagonizing effect of chloramphenicol on fosfomycin-induced lysis (Mengin-Lecreux & van Heijenoort, 1990). The biochemical explanation provided was that the increase of the UDP-GlcNAc pool modulates the residual transferase activity not inhibited by fosfomycin, thus ensuring the formation of enough UDP-MurNAc-pentapeptide to sustain peptidoglycan synthesis at a rate that will antagonize lysis. The determination of the level of residual racemase and ligase activities in D-cycloserine-treated cells could perhaps provide a similar explanation for the effect of chloramphenicol in this case.

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