JOURNAL OF BACTERIOLOGY, Sept. 1978, p. 766-774
0021-9193/78/0135-0766$02.00/0
Copyright © 1978 American Society for Microbiology
Vol. 135, No. 3
Printed in U.S.A.

Involvement of the *relA* Gene Product and Feedback Inhibition in the Regulation of UDP-*N*-Acetylmuramyl-Peptide Synthesis in *Escherichia coli*

EDWARD E. ISHIGURO†* AND WILLIAM D. RAMEY

Department of Microbiology, University of British Columbia, Vancouver, British Columbia, V6T 1W5, Canada

Received for publication 8 May 1978

The regulation of uridine diphosphate-*N*-acetylmuramyl-peptide (UDP-MurNAc-peptide) synthesis was studied by labeling *Escherichia coli* strains auxotrophic for lysine and diaminopimelate with [^{3}H]diaminopimelate for 15 min under various conditions. The amounts of [^{3}H]diaminopimelate incorporated into UDP-MurNAc-tripeptide and -pentapeptide by a stringent (rel^+) strain were the same in the presence or absence of lysine. Chloramphenicol-treated rel^+ cells showed a 2.8-fold increase in labeled UDP-MurNAc-pentapeptide. An isogenic relaxed (*relA*) strain deprived of lysine showed a 2.7-fold increase in UDP-MurNAc-pentapeptide. Thus, UDP-MurNAc-pentapeptide synthesis is regulated by the *relA* gene. D-Cycloserine treatment of rel^+ and *relA* strains caused a depletion of intracellular UDP-MurNAc-pentapeptide. Labeled UDP-MurNAc-tripeptide accumulated in D-cycloserine-treated cells of the rel^+ and *relA* strains, suggesting that UDP-MurNAc-pentapeptide is a feedback inhibitor of UDP-MurNAc-peptide synthesis. In lysine-deprived cells, D-cycloserine treatment caused 41- and 71-fold accumulations of UDP-MurNAc-tripeptide in rel^+ and *relA* strains, respectively. A 124-fold increase in UDP-MurNAc-tripeptide occurred in lysine-deprived rel^+ cells treated with both chloramphenicol and D-cycloserine. These results indicate that both the *relA* gene product and feedback inhibition are involved in regulating UDP-MurNAc-peptide synthesis during amino acid deprivation.

Peptidoglycan is a polymer found in the cell walls of most bacteria. Figure 1 summarizes some of the major steps in the synthesis of peptidoglycan in *Escherichia coli*. There are three stages in peptidoglycan synthesis, each of which occurs in a different cellular location (3). In the first stage, the nucleotide precursors, UDP-*N*-acetylglucosamine (UDP-GlcNAc) and UDP-*N*-acetylmuramyl-L-Ala-D-Glu-*meso*-diaminopimelic acid-D-Ala-D-Ala (UDP-MurNAc-pentapeptide), are synthesized by a series of soluble enzymes (steps 1 to 8). The second stage is the translocation of MurNAc-pentapeptide (step 9) and GlcNAc (step 10) to a membrane glycosyl carrier lipid (GCL-P) by particulate enzymes to form the lipid intermediates, GCL-P-P-MurNAc-pentapeptide and GCL-P-P-MurNAc (-GlcNAc)-pentapeptide. In the third stage, MurNAc (-GlcNAc)-pentapeptide is transferred to an acceptor site in the growing peptidoglycan polymer (step 11), and the resulting GCL-P-P is dephosphorylated to yield GCL-P (step 12).

We have shown that the synthesis of peptidoglycan in *E. coli* is under stringent control (4). When stringent (rel^+) strains were deprived of a required amino acid, the incorporation of radioactive diaminopimelic acid (DAP) into peptidoglycan was inhibited. In contrast, the synthesis of peptidoglycan in relaxed (*relA*) mutants was unaffected by amino acid deprivation. Guanosine 5′-triphosphate 3′-diphosphate (pppGpp) and guanosine 5′-diphosphate 3′-diphosphate (ppGpp) accumulate during amino acid deprivation in rel^+ strains but not in *relA* strains, and these nucleotides may be the mediators of stringent control (1). The accumulation of pppGpp and ppGpp does not occur during treatment with chloramphenicol (CAM), and stringent control is abolished. Thus, the synthesis of peptidoglycan was relaxed when rel^+ bacteria were treated with CAM in the presence or absence of required amino acids (4). We have demonstrated that the incorporation of MurNAc (-GlcNAc)-pentapeptide into acceptor peptido-

† Present address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., V8W 2Y2, Canada.

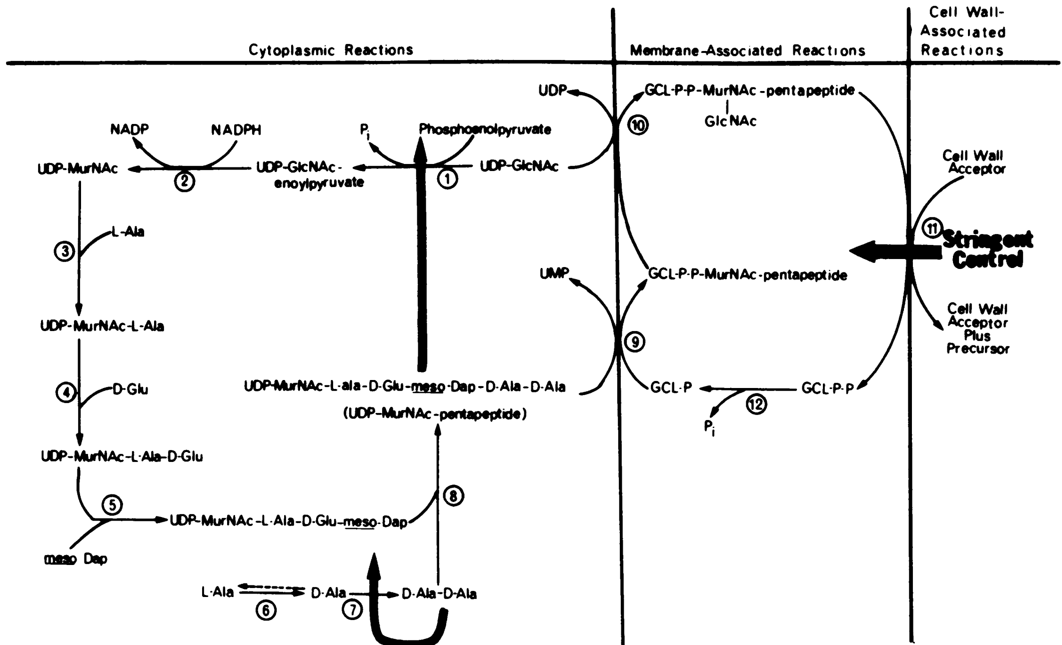

FIG. 1. *Major steps in peptidoglycan biosynthesis in E. coli. Large, barred arrow through reaction 11 designates site of stringent control. Thick, solid arrows through reactions 1 and 7 indicate sites thought to be regulated by feedback inhibition. Broken arrow in reaction 6 indicates that reaction is influenced by the ratio of L-Ala to D-Ala. See text for details.*

glycan was inhibited in amino acid-deprived *rel*⁺ bacteria; the synthesis of nucleotide precursors and lipid intermediates was not inhibited during amino acid deprivation (10). Therefore, reaction 11 in Fig. 1 is the site of stringent control.

In *E. coli*, the rate of nucleotide precursor synthesis appears to be coupled to the rate at which the precursors are utilized for the synthesis of peptidoglycan. For example, UDP-MurNAc-pentapeptide did not accumulate when the membrane-bound reactions in peptidoglycan synthesis were inhibited by incubating temperature-sensitive mutants at a nonpermissive temperature (8). Furthermore, nucleotide precursors did not accumulate when the synthesis of peptidoglycan was inhibited by treatment with penicillin or vancomycin (8). However, treatment of cells with D-cycloserine (DCS) resulted in an accumulation of UDP-MurNAc-L-Ala-D-Glu-*meso*-DAP (UDP-MurNAc-tripeptide). It was shown (6) that DCS prevented the synthesis of D-Ala-D-Ala (and presumably, UDP-MurNAc-pentapeptide) by inhibiting alanine racemase (step 6, Fig. 1) and D-Ala-D-Ala synthetase (step 7, Fig. 1). These results suggested that UDP-MurNAc-pentapeptide may regulate its own synthesis, possibly by feedback inhibiting phosphoenolpyruvate:UDP-GlcNAc enolpyruvyltransferase, the enzyme catalyzing reaction 1 in Fig. 1 (8, 11). The following mechanisms may also be involved in regulating the intracellular levels of nucleotide precursors. (i) D-Ala-D-Ala synthetase (step 7, Fig. 1) is inhibited by D-Ala-D-Ala (6), and this may be important in regulating the level of D-Ala-D-Ala. (ii) Alanine racemase (step 6, Fig. 1) exhibits a higher K_m value for L-Ala than for D-Ala, and the V_{max} value for the anabolic reaction (L-Ala → D-Ala) is higher than the V_{max} value for the catabolic reaction (D-Ala → L-Ala). Thus, the synthesis of D-Ala is favored only when the intracellular ratio of L-Ala to D-Ala is high. (iii) Accumulation of D-Ala may result in the repression of alanine racemase synthesis (2, 5) and the induction of alanine dehydrogenase synthesis (2).

In this paper, we present evidence indicating that the synthesis of UDP-MurNAc-peptides during amino acid deprivation in *E. coli* is regulated by (i) the *relA* gene product and (ii) feedback inhibition of an unidentified enzyme by UDP-MurNAc-pentapeptide.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* K-12 strains used in this study have been described previously (4). Strain LD5456 (*relA lysA dapD thi*) is an isogenic derivative of strain LD5 (*rel*⁺ *lysA dapD thi*).

Labeling of bacteria. Bacterial cultures were grown at 37°C in M9 minimal medium with 0.4% glucose and required growth factors as described previously (4). Two types of experiments were performed. In the first series of experiments, we tested the effects of amino deprivation, treatment with CAM, and treatment with DCS on the incorporation of [^{3}H]DAP into nucleotide precursors and peptidoglycan. Bacteria were grown for four doublings to a density of 2×10^8 to 3×10^8 cells per ml. The cells were then harvested and washed as described previously (4). The washed cells were inoculated into 10-ml quantities of M9 medium containing 0.2 μg of [^{3}H]DAP (300 μCi/μmol) per ml. The effects of CAM and DCS on the synthesis of nucleotide precursors and peptidoglycan were determined by adding CAM (200 μg/ml) and DCS (40 μg/ml) to the medium. Amino acid deprivation was achieved by omitting lysine from the medium. In the second series of experiments, bacteria were prelabeled and then subjected to the various treatments in the absence of an exogenous supply of DAP. Bacteria were grown in M9 medium containing 0.2 μg of [^{3}H]DAP (300 μCi/μmol) per ml for 60 min. The labeled cells were harvested and washed. Washed cells were subjected to lysine deprivation, CAM treatment, and DCS treatment in 10-ml quantities of M9 medium lacking DAP. We determined the effects of the various treatments on the incorporation of labeled intracellular intermediates into peptidoglycan and on the intracellular levels of labeled nucleotide precursors. In both types of experiments, cultures were incubated in a 37°C water bath shaker, and the synthesis of peptidoglycan was determined by monitoring the incorporation of radioactivity into trichloroacetic acid-insoluble material as described previously (4).

Determination of labeled nucleotide precursors. Labeled UDP-MurNAc-tripeptide and UDP-MurNAc-pentapeptide were quantitated by paper chromatography by previously described methods (7, 8). At designated times, 8-ml samples were removed from labeled cultures and centrifuged for 3 min at $35{,}000 \times g$ in a Sorvall RC2B centrifuge operating at 2°C. The culture supernatants were saved for analysis of autolytic products and excreted nucleotide precursors. The cell pellets were resuspended in 60 μl of cold, distilled water containing unlabeled reference nucleotide precursors in quantities sufficient to be detected by short-wave UV light (8). The samples of cells were applied quantitatively as 1-cm streaks to Whatman 3MM chromatography paper (57 cm long), and the chromatogram was developed in isobutyric acid-1 M NH_4OH (5:3) for 62 h. Radioactive spots were detected and counted as previously described (4, 8). The radioactivities of the nucleotide precursors were normalized to cell mass. They are expressed as counts per minute per milligram of cell dry weight. The dry weight of cells applied to the chromatogram was determined turbidimetrically from a standard curve (4).

The culture supernatants were concentrated by lyophilization and desalted by gel filtration on a column (2.5 by 50 cm) of Sephadex G-25. The desalted samples were lyophilized and analyzed for the presence of labeled autolytic products and nucleotide precursors by paper chromatography as described above.

Materials. [G-^{3}H]DAP was purchased from the Amersham/Searle Corp. CAM and DCS were obtained from the Sigma Chemical Co. Unlabeled nucleotide precursors were prepared as previously described (4, 8).

RESULTS

The effects of inhibition of protein synthesis on the incorporation of [^{3}H]DAP into nucleotide precursors were compared in strains LD5 (*rel*$^+$) and LD5456 (*relA*) (data not shown). An eightfold accumulation of labeled nucleotide precursors occurred when strain LD5456 (*relA*) was deprived of lysine for 60 min. Under identical conditions, strain LD5 (*rel*$^+$) did not accumulate nucleotide precursors. However, nucleotide pre-

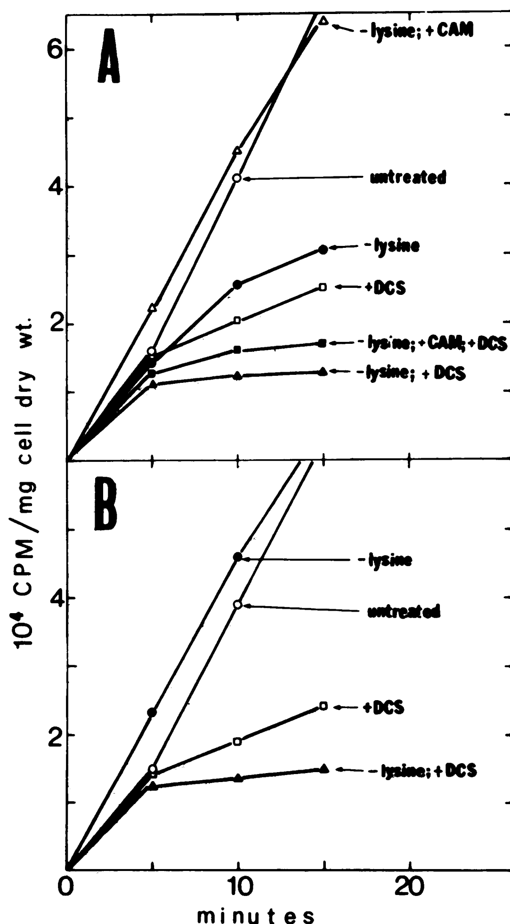

FIG. 2. *Incorporation of [^{3}H]DAP into peptidoglycan by strains LD5 rel$^+$ (A) and LD5456 relA (B) under various conditions. Washed cells were inoculated into M9* (○), *M9 without lysine* (●), *M9 without lysine but with CAM* (△), *M9 with DCS* (□), *M9 without lysine but with DCS* (▲), *and M9 without lysine but with CAM and DCS* (■).

cursors accumulated by over fivefold when strain LD5 (*rel*$^{+}$) was treated with CAM for 60 min in the presence or absence of lysine. UDP-MurNAc-pentapeptide was the major labeled component in the nucleotide precursor pools of both strains under all conditions tested. At least 87% of the total radioactivity incorporated into nucleotide precursors was present in this component. These results indicate that the product of the *relA* gene is involved in preventing the accumulation of nucleotide precursors, particularly UDP-MurNAc-pentapeptide, in amino acid-deprived bacteria. It should be noted that in all experiments described in this paper, cell lysis or leakage was not apparent, and labeled nucleotide precursors were not detected in culture supernatants.

Two types of experiments were performed with CAM, DCS, and lysine deprivation to evaluate the roles of the *relA* gene product and feedback inhibition in the regulation of nucleotide precursor synthesis. In these experiments, DCS was used at a concentration of 40 μg/ml. At this concentration, DCS did not affect growth (increase in cell mass) of strains LD5 (*rel*$^{+}$) and LD5456 (*relA*) for at least 10 to 15 min. Longer periods of treatment resulted in a decrease in growth rate, and the onset of cell lysis (determined by leakage of labeled nucleotide precursors) was observed after approximately 20 min of treatment. As shown previously (4), growth of both strains ceased whenever protein synthesis was inhibited.

In the first series of experiments, the effects of various treatments on the incorporation of [^{3}H]DAP into peptidoglycan and into nucleotide precursors was determined in strains LD5 (*rel*$^{+}$) and LD5456 (*relA*). The following conclusions can be derived from Fig. 2. (i) During lysine deprivation, the synthesis of peptidoglycan was stringently controlled in strain LD5 (*rel*$^{+}$) but was relaxed in strain LD5456 (*relA*). (ii) Peptidoglycan synthesis in strain LD5 (*rel*$^{+}$) was relaxed when CAM was present during lysine deprivation. (iii) In both strains, the incorporation of [^{3}H]DAP into peptidoglycan was inhibited whenever the cells were treated with DCS. The intracellular levels of labeled nucleotide precursors synthesized under these conditions by strains LD5 (*rel*$^{+}$) and LD5456 (*relA*) are shown

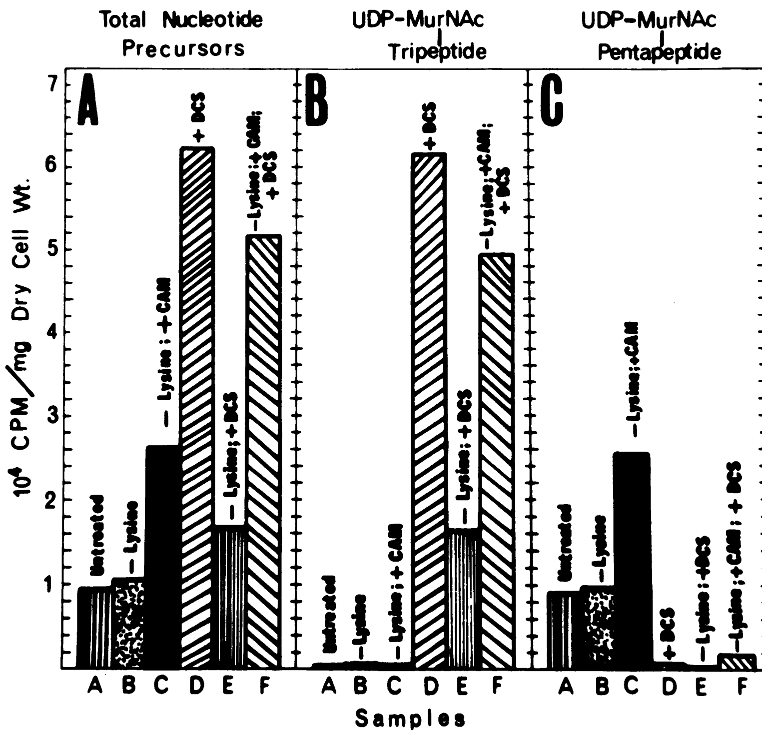

FIG. 3. *Effects of various treatments on the intracellular levels of total nucleotide precursors (A), UDP-MurNAc-tripeptide (B), and UDP-MurNAc-pentapeptide (C) in strain LD5 rel*$^{+}$*. The levels of labeled nucleotide precursors were determined in cells which were treated for 15 min as described in the legend to Fig. 2A. The sources of the samples were: sample A, M9; sample B, M9 without lysine; sample C, M9 without lysine but with CAM; sample D, M9 with DCS; sample E, M9 without lysine but with DCS; and sample F, M9 without lysine but with CAM and DCS.*

in Fig. 3 and 4, respectively. The important points are as follows. (i) In strain LD5 (*rel*$^+$), the amounts of [^{3}H]DAP incorporated into nucleotide precursors were approximately equal in the presence and absence of lysine, but treatment with CAM resulted in a 2.8-fold increase in the level of labeled nucleotide precursors. A 2.7-fold increase in labeled nucleotide precursors occurred when strain LD5456 (*relA*) was deprived of lysine. In all cases, UDP-MurNAc-pentapeptide accounted for at least 91% of the total labeled nucleotide precursors. These results demonstrate the involvement of the *relA* gene product in the regulation of nucleotide precursor synthesis. (ii) In both strains, labeled nucleotide precursors always accumulated whenever cells were treated with DCS. Under all conditions, DCS clearly prevented the synthesis of UDP-MurNAc-pentapeptide. UDP-MurNAc-tripeptide accounted for at least 98% of the labeled nucleotide precursors accumulated in the presence of DCS. This explains the inhibitory effect of DCS on the incorporation of [^{3}H]DAP into peptidoglycan (Fig. 2). Furthermore, if the bacteria continued to utilize preexisting (unlabeled) UDP-MurNAc-pentapeptide during treatment with DCS, the supply of this nucleotide precursor would decrease progressively. The accumulation of labeled UDP-MurNAc-tripeptide observed during this period supports the proposal (8) that UDP-MurNAc-pentapeptide controls the synthesis of nucleotide precursors by feedback inhibition. (iii) As shown in Fig. 3, a combination of DCS treatment and lysine deprivation in strain LD5 (*rel*$^+$) resulted in a 1.8-fold accumulation of labeled nucleotide precursors. However, labeled nucleotide precursors accumulated to an even greater degree (5.4-fold) when lysine deprivation and treatment with DCS and CAM were combined. Furthermore, as shown in Fig. 4, lysine deprivation of strain LD5456 (*relA*) resulted in a 2.7-fold increase in labeled nucleotide precursors, but a 6.4-fold accumulation occurred when lysine deprivation was combined with DCS treatment. Together, these results indicate that both feedback inhibition and the *relA* gene product are involved in regulating the synthesis of nucleotide precursors

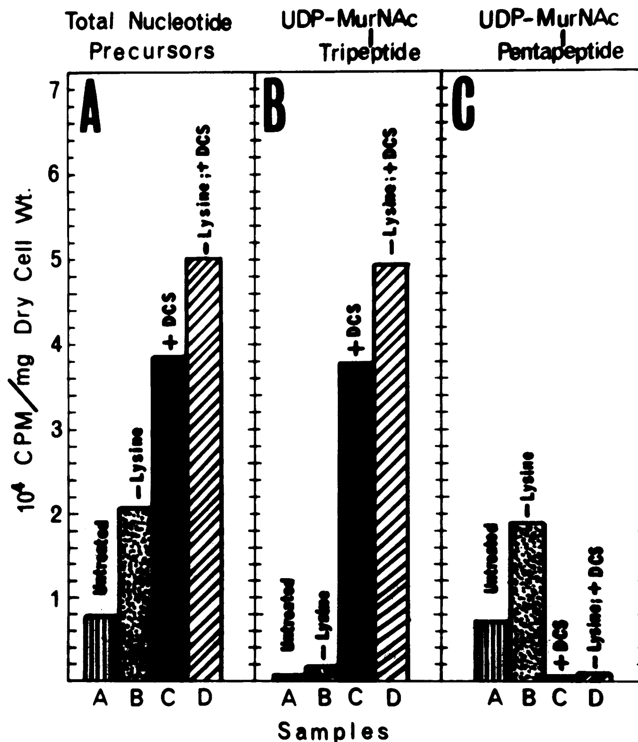

FIG. 4. *Effects of various treatments on the intracellular levels of total nucleotide precursors (A), UDP-MurNAc-tripeptide (B), and UDP-MurNAc-pentapeptide (C) in strain LD5456 relA. The levels of labeled nucleotide precursors were determined in cells which were treated for 15 min as described in the legend to Fig. 2B. The sources of the samples were: sample A, M9; sample B, M9 without lysine; sample C, M9 with DCS; sample D, M9 without lysine but with DCS.*

during amino acid deprivation.

In the second series of experiments, bacteria were first labeled with [^{3}H]DAP and then subjected to various treatments in M9 medium lacking DAP. Under these conditions, strains LD5 (*rel*$^+$) and LD5456 (*relA*) have a sufficient intracellular supply of DAP and other intermediates for peptidoglycan synthesis to support growth for at least 120 min, i.e., two doublings (4). The effects of various treatments on the incorporation of labeled intracellular intermediates into peptidoglycan by strains LD5 (*rel*$^+$) and LD5456 (*relA*) are shown in Fig. 5. The utilization of labeled precursors during lysine deprivation was stringently controlled in strain LD5 (*rel*$^+$) but was relaxed in strain LD5456 (*relA*). The control was also relaxed when strain LD5 (*rel*$^+$) was deprived of lysine in the presence of CAM. In both strains, the incorporation of labeled precursors into peptidoglycan decreased with time when cells were treated with DCS. The intracellular levels of labeled nucleotide precursors in strains LD5 (*rel*$^+$) and LD5456 (*relA*) before treatment and after 10 min of various treatments are shown in Fig. 6 and 7. The following results are similar to those from the first series of experiments described above. (i) The levels of nucleotide precursors in strain LD5 (*rel*$^+$) were not altered during lysine deprivation. However, UDP-MurNAc-pentapeptide accumulated over twofold when strain LD5456 (*relA*) was deprived of lysine or when strain LD5 (*rel*$^+$) was deprived of lysine in the presence of CAM. (ii) DCS treatment alone, or in combination with other treatments, caused an accumulation of UDP-MurNAc-tripeptide in both strains. (iii) DCS caused a maximum accumulation of UDP-MurNAc-tripeptide in lysine-deprived bacteria under conditions where the *relA* gene product was rendered nonfunctional, i.e., in strain LD5456 (*relA*) or in strain LD5 (*rel*$^+$) in the presence of CAM. In Fig. 6 and 7, it is important to note that DCS inhibited the synthesis of UDP-MurNAc-pentapeptide, but it did not prevent the utilization of pre-existing UDP-MurNAc-pentapeptide. Therefore, the levels of labeled UDP-MurNAc-pentapeptide decreased by at least 75% during treatment with DCS. This was coincident with the accumulation of labeled UDP-MurNAc-tripeptide. Collectively, these results support the conclusion that both feedback inhibition and the *relA* gene product are involved in preventing the accumulation of UDP-MurNAc-pentapeptide during amino acid deprivation.

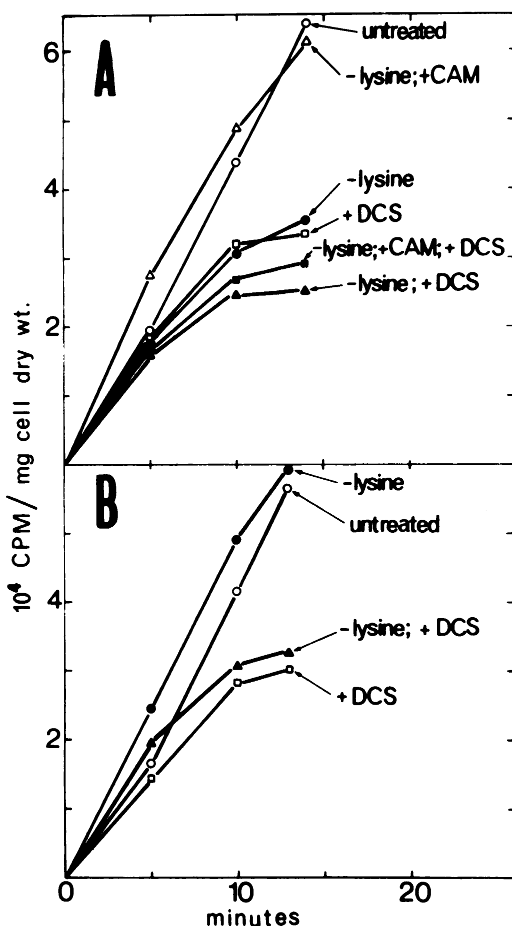

FIG. 5. *Effects of various treatments on the incorporation of labeled intracellular intermediates into peptidoglycan by strains LD5 rel$^+$ (A) and LD5456 relA (B). Cells labeled with [^{3}H]DAP were harvested, washed, and inoculated into the following media without an exogenous supply of DAP: M9 (○), M9 without lysine (●), M9 without lysine but with CAM (△), M9 with DCS (□), M9 without lysine but with DCS (▲), and M9 without lysine but with CAM and DCS (■). Utilization of labeled intracellular intermediates was determined by following the incorporation of radioactivity into trichloroacetic acid-insoluble fractions. The radioactivities present in peptidoglycan at zero time have been subtracted from the data (19.3 × 10^4 and 18.2 × 10^4 cpm/mg of cell dry weight for LD5 and LD5456, respectively).*

DISCUSSION

During amino acid deprivation of *E. coli*, the activities of the soluble and the particulate enzymes involved in peptidoglycan biosynthesis are regulated independently. We have already presented evidence indicating that the translocation of MurNAc (-GlcNAc)-pentapeptide into nascent peptidoglycan (reaction 11, Fig. 1) is inhibited in *rel*$^+$ bacteria but not in *relA* mutants (10). The results presented above show that both feedback inhibition and the *relA* gene product

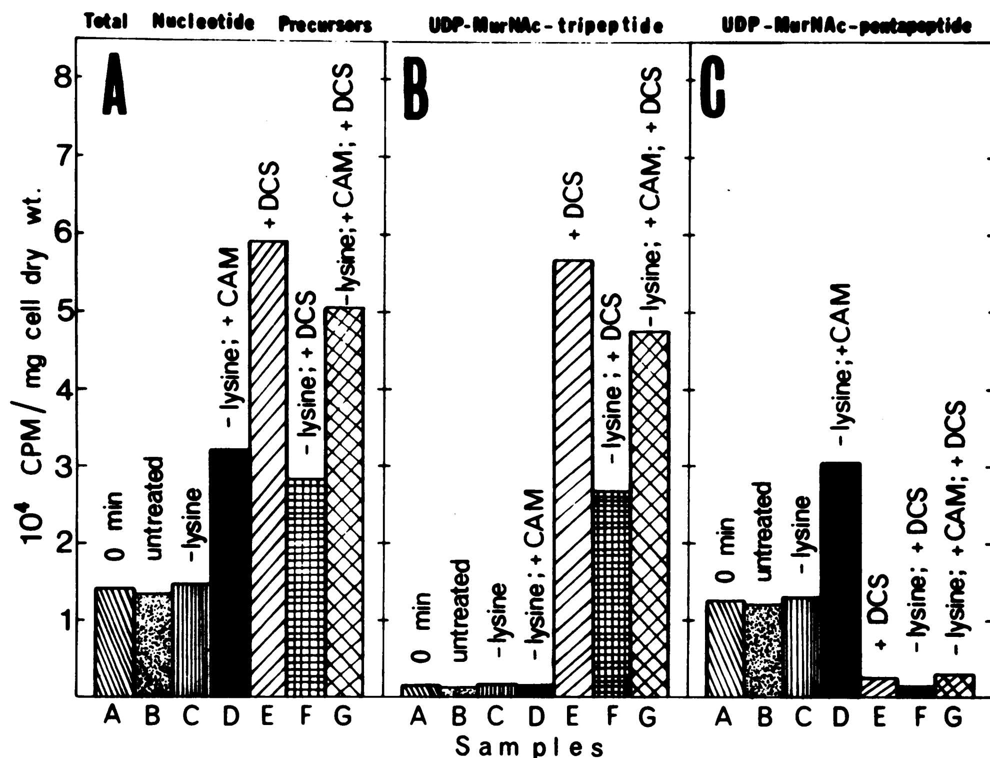

FIG. 6. *Effects of various treatments on the intracellular levels of total nucleotide precursors (A), UDP-MurNAc-tripeptide (B), and UDP-MurNAc-pentapeptide (C) in prelabeled cells of strain LD5 rel⁺. Cells were prelabeled with [^{3}H]DAP and then treated in the absence of DAP as described in the legend to Fig. 5A. The levels of labeled nucleotide precursors were determined in samples of cells collected before treatment (sample A) and after 10 min of incubation in the following media: M9 (sample B), M9 without lysine (sample C), M9 without lysine but with CAM (sample D), M9 with DCS (sample E), M9 without lysine but with DCS (sample F), and M9 without lysine but with CAM and DCS (sample G).*

are involved in regulating the synthesis of nucleotide precursors. We have not yet identified the site(s) of regulation in the soluble-enzyme system. The simplest and perhaps most appealing model would be one in which both controls exert their effects on the activity of a single enzyme.

The conclusion derived from our experiments with DCS is in agreement with that of Lugtenberg et al. (8) who proposed that UDP-MurNAc-pentapeptide regulated its own synthesis by feedback inhibition. Venkateswaran et al. (11) demonstrated that UDP-MurNAc-pentapeptide inhibited the in vitro activity of phosphoenolpyruvate: UDP-GlcNAc enolpyruvyltransferase, the enzyme catalyzing reaction 1 in Fig. 1. Although this enzyme is the most logical site for feedback inhibition, the observation was difficult to interpret because UDP-MurNAc-pentapeptide also inhibited the in vitro activity of the same enzyme obtained from *Bacillus cereus.* Unlike *E. coli, B. cereus* accumulates UDP-MurNAc-pentapeptide when peptidoglycan synthesis is inhibited by antibiotics. It appears necessary to determine the regulatory site in *E. coli* in vivo because it is often difficult to correlate in vitro results with in vivo phenomena.

We have two comments to make concerning the experiments performed by Lugtenberg et al. (8). First, they studied the synthesis of nucleotide precursors and peptidoglycan in a cell wall synthesis medium designated as CWSM-I. CWSM-I contained glucose, salts, L-[^{14}C]alanine, L-glutamic acid, DAP, uracil, and CAM (7). Bacterial strains requiring amino acids not present in CWSM-I were used in virtually all of their experiments. Therefore, they were determining the incorporation of L-[^{14}C]alanine into nucleotide precursors and peptidoglycan by bacteria which were subjected to both amino acid deprivation and CAM treatment. According to our results, the synthesis of both peptidoglycan and nucleotide precursors should be relaxed under these conditions. Lugtenberg et al. failed to

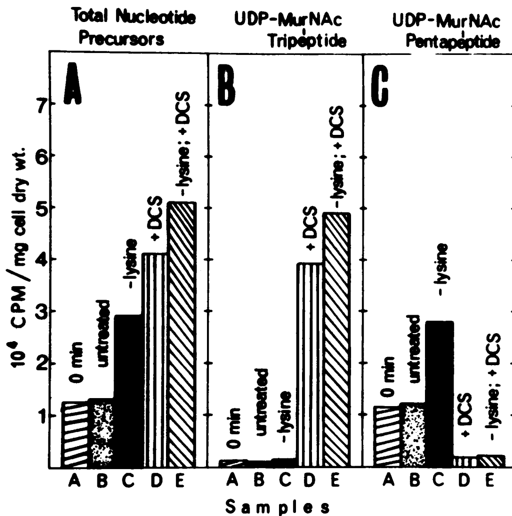

FIG. 7. *Effects of various treatments on the intracellular levels of total nucleotide precursors (A), UDP-MurNAc-tripeptide (B), and UDP-MurNAc-pentapeptide (C) in prelabeled cells of strain LD5456 relA. Cells were prelabeled with [^{3}H]DAP and then treated in the absence of DAP as described in the legend to Fig. 5B. The levels of labeled nucleotide precursors were determined in samples of cells collected before treatment (sample A) and after 10 min of incubation in the following media: M9 (sample B), M9 without lysine (sample C), M9 with DCS (sample D), and M9 without lysine but with DCS (sample E).*

make this observation because control cultures which were not subjected to amino acid deprivation and to CAM treatment were never included in their experiments. Our second comment concerns the effect of DCS on the synthesis of nucleotide precursors. In agreement with our results, Lugtenberg et al. (8) reported that UDP-MurNAc-tripeptide was the major nucleotide precursor synthesized in the presence of DCS. However, they found that the total amount of nucleotide precursors synthesized by bacteria treated with DCS in CWSM-I was only 29% of the amount synthesized by control bacteria in CWSM-I without DCS. Our results show that DCS-treated bacteria consistently had higher levels of nucleotide precursors than bacteria which were not treated with DCS. For example, when strain LD5 (*rel*$^+$) was subjected to lysine deprivation, CAM treatment, and DCS treatment (sample F, Fig. 3A), the total amount of nucleotide precursors was almost twofold higher than the amount in cells subjected to only lysine deprivation and CAM treatment (sample C, Fig. 3A). It should be noted that the conditions in this example most closely resemble the experimental conditions employed by Lugtenberg et al. The low levels of nucleotide precursors recovered from DCS-treated bacteria by Lugtenberg et al. could have been due to lysis of the cells during the period of treatment (60 min). DCS eventually causes cell lysis even when protein synthesis is inhibited (Ishiguro, unpublished data). In conclusion, the results of Lugtenberg et al. do not adequately demonstrate a correlation between the accumulation of UDP-MurNAc-tripeptide and a decrease in the intracellular level of UDP-MurNAc-pentapeptide. This correlation is clearly established in the experiments described in this paper.

As noted above, nucleotide precursors accumulate when peptidoglycan synthesis is inhibited in some bacteria, notably *Staphylococcus aureus* (9) and *B. cereus* (11). Why these organisms differ from *E. coli* in this respect is not known. It may be worthwhile to determine whether gram-negative bacteria generally exhibit feedback inhibition and whether the lack of this control is widespread among gram-positive bacteria.

Under our experimental conditions, DCS treatment by itself did not affect bacterial growth for at least 10 to 15 min. The accumulation of UDP-MurNAc-tripeptide during this period suggests that feedback inhibition regulates nucleotide precursor synthesis in growing cells. Because nucleotide precursors accumulated in lysine-deprived bacteria treated with DCS, feedback inhibition is also operative in nongrowing cells. Although the results of this study are insufficient to permit an assessment of the regulatory role of the *relA* gene during growth, we have clearly demonstrated that feedback inhibition alone is insufficient to regulate nucleotide precursor synthesis during amino acid deprivation and that the *relA* gene control is also required. It is worthwhile considering the possibility that regulation by feedback inhibition alone may be sufficient during steady-state growth. If this is so, the *relA* gene product may be viewed as an accessory control which is put into effect by bacteria during any nutritional downshift resulting in accumulations of ppGpp and pppGpp. It is evident from our findings that the *relA* gene control is necessary to readjust the rate of nucleotide precursor synthesis in bacteria subjected to a severe downshift such as amino acid deprivation.

ACKNOWLEDGMENTS

W. D. Ramey was the recipient of a Frank Wesbrook predoctoral fellowship from the University of British Columbia. This investigation was supported by grant A6606 from the National Research Council of Canada.

LITERATURE CITED

1. Cashel, M. 1975. Regulation of bacterial ppGpp and

pppGpp. Annu. Rev. Microbiol. **29**:301–318.

2. **Franklin, F. C. H., and W. A. Venables.** 1976. Biochemical, genetic, and regulatory studies of alanine catabolism in *Escherichia coli* K-12. Mol. Gen. Genet. **149**:229–237.
3. **Ghuysen, J. M., and G. D. Shockman.** 1973. Biosynthesis of peptidoglycan, p. 37–130. *In* L. Lieve (ed.), Bacterial membranes and walls. Marcel Dekker, Inc., New York.
4. **Ishiguro, E. E., and W. D. Ramey.** 1976. Stringent control of peptidoglycan biosynthesis in *Escherichia coli* K-12. J. Bacteriol. **127**:1119–1126.
5. **Lambert, M. P., and F. C. Neuhaus.** 1971. Factors affecting the level of alanine racemase in *Escherichia coli*. J. Bacteriol. **109**:1156–1161.
6. **Lugtenberg, E. J. J.** 1972. Studies on *Escherichia coli* enzymes involved in the synthesis of uridine diphosphate-*N*-acetyl-muramyl-pentapeptide. J. Bacteriol. **110**:26–34.
7. **Lugtenberg, E. J. J., and P. G. De Haan.** 1971. A simple method for following the fate of alanine-containing components in murein synthesis in *Escherichia coli*. Antonie van Leeuwenhoek J. Microbiol. Serol. **37**: 537–552.
8. **Lugtenberg, E. J. J., L. De Haas-Menger, and W. H. M. Ruyters.** 1972. Murein synthesis and identification of cell wall precursors of temperature-sensitive lysis mutants of *Escherichia coli*. J. Bacteriol. **109**:326–335.
9. **Park, J. T., and M. J. Johnson.** 1949. Accumulation of labile phosphate in *Staphylococcus aureus* grown in the presence of penicillin. J. Biol. Chem. **179**:585–592.
10. **Ramey, W. D., and E. A. Ishiguro.** 1978. Site of inhibition of peptidoglycan biosynthesis during the stringent response in *Escherichia coli*. J. Bacteriol. **135**:71–77.
11. **Venkateswaran, P. S., E. J. J. Lugtenberg, and H. C. Wu.** 1973. Inhibition of phosphoenolpyruvate: uridine diphosphate *N*-acetylglucosamine enolpyruvyltransferase by uridine diphosphate *N*-acetylmuramyl peptides. Biochim. Biophys. Acta **293**:570–574.