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Identification of an Outer Membrane Protein of *Escherichia coli*, with a Role in the Coordination of Deoxyribonucleic Acid Replication and Cell Elongation

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Protein G of molecular weight 15,000 is the fourth commonest protein in the outer membrane of *Escherichia coli* B/r. From experiments described here on the relationship of protein G production to cell elongation and septation, the hypothesis is proposed that protein G is a structural protein of cell elongation. Furthermore, a surplus of protein G is produced when deoxyribonucleic acid synthesis is arrested and septation is thereby prevented. Thus protein G may be an important coordination protein in *E. coli* for integration of deoxyribonucleic acid synthesis, cell envelope elongation, and septation. Inhibition of normal cell elongation in a rod configuration in *E. coli* B/r by the novel amidinopenicillanic acid FL1060 was accompanied by changes in the rate of appearance of protein G and several other outer membrane proteins. The rate of appearance of protein G decreased some 70% within 60 min, in parallel with termination of rounds of normal cell elongation. Filament-inducing concentrations of nalidixic acid increased dramatically the rate of appearance of protein G. After 30 min a plateau level some 250% higher than the control value was reached. Similar kinetics were observed in parallel with filament formation induced by incubation of a *dnaB* mutant of *E. coli* at the nonpermissive temperature. No change in the rate of appearance of protein G was observed during cephalexin- or benzylpenicillin-induced filament formation, indicating that increased protein G production was not a secondary consequence of filamentation. Cells treated with FL1060 lost their ability to be induced for protein G formation, with nalidixic acid, in parallel with their loss of ability to initiate rounds of normal cell elongation. A pulse-chase experiment demonstrated that the protein G appearing in the outer membrane as a consequence of inhibition of deoxyribonucleic acid synthesis was the result of de novo synthesis rather than of interconversion from previously synthesized protein species. A preliminary characterization of protein G revealed several similarities with the well-characterized lipoprotein of the outer membrane of *E. coli*. A comparison of the incorporation of several ^{14}C-labeled amino acids into protein G and the lipoprotein revealed substantial differences, however, perhaps ruling out a simple relationship between these two proteins.

Cell shape and the process of cell division in *Escherichia coli* are dependent upon the biochemistry of the cell envelope, consisting of a cytoplasmic membrane, a rigid murein layer, and an outer membrane (2, 14, 23). The synthesis of the cell envelope is of two main kinds—elongation and septation. These processes can be resolved by the use of β-lactam antibiotics, which specifically inhibit murein synthesis.

One class of murein synthesis appears to be necessary for cell elongation in a rod configuration. The initiation of this process, at an early stage of the cell cycle, is specifically inhibited by a novel amidinopenicillanic acid, FL1060, with the result that cells grow into osmotically stable spheres (20). FL1060 binds specifically to a very small number of molecules (about 10 per cell) of an *E. coli* inner membrane protein (28). The small quantity of this protein, its inner membrane location, and its periodic function to initiate elongation in a rod configuration suggest that it acts enzymatically to create new initiation structures in the murein, rather than as a structural membrane protein.

A second class of murein synthesis, which occurs late in the *E. coli* cell cycle and is required for septation, is inhibited specifically by low concentrations of benzylpenicillin (16) or by cephalexin (20), resulting in the formation of multinucleate, aseptate filaments. These anti-

biotics bind relatively specifically to another inner membrane protein (27). Septation is also dependent upon continued deoxyribonucleic acid (DNA) synthesis (8, 18); treatments that inhibit DNA synthesis, such as nalidixic acid, ultraviolet irradiation, thymine starvation of a thymine auxotroph, or incubation of a *dnaB* mutant at the nonpermissive temperature, block septation and yet allow cell elongation to proceed.

If elongation is indeed a different biochemical process than septation, one should be able to find biochemical differences between cells that are elongating and those that are septating. To separate these two processes, we have utilized specific penicillins and inhibitors of DNA synthesis as selective inhibitors. FL1060 applied to an exponential culture of *E. coli* B/r converts envelope growth during one cell cycle from a combination of septation and elongation to a pure septation pattern. In contrast, cephalexin, low concentrations of benzylpenicillin, or inhibitors of DNA synthesis allow only the elongation process. Under these two conditions one can examine the cell envelope and see whether any proteins present in relatively large amounts (structural proteins) are made at different rates. We have restricted our search for such proteins to the outer membrane of *E. coli* B/r, a structure having a much less complex protein composition than the inner membrane but becoming more and more recognized as an important component of the cellular apparatus for shape determination (13, 14). Many attempts have been made to find changes in the murein composition of cells of different morphology; however, as yet this has proven a rather fruitless search.

In this paper we report the identification of a major outer membrane protein (protein G) that appears to satisfy the basic requirements for a structurally component of cell elongation. It is a protein with an apparent molecular weight of 15,000 with properties similar to those of the well-characterized 7,200-molecular-weight lipoprotein, that exists in a free form in the *E. coli* outer membrane (4, 5) or in a bound form in which it is covalently linked to murein (6).

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* B/r (laboratory collection) and *E. coli* B/r *thy⁻ his⁻* (obtained from C. E. Helmstetter) were routinely grown at 37 C with shaking in M9 minimal salts-glucose medium supplemented with histidine (50 μg/ml) or thymine (20 μg/ml) as required. *E. coli* MX74 T2 and its *dnaB* derivative, *E. coli* MX74 T2 ts27 (laboratory collection), were grown at 30 C in supplemented M9 minimal salts-glucose medium and transferred to 42 C for study of the restrictive phenotype.

Determination of cell number. A formalin-saline solution consisting of 50 ml of 38% formaldehyde and 9.0 g of NaCl per liter of distilled water was filtered through a 0.22-μm membrane filter. Samples of cultures were diluted into 10 ml of formalin-saline and the cell number was determined with a Coulter counter model B.

Radioactive labeling and membrane preparation. In most experiments, 5-ml samples of exponentially growing cultures of *E. coli* were pulsed with 5 μCi of [^{35}S]methionine per ml (200 Ci/mmol, New England Nuclear Corp.) for 3 min. Incorporation was terminated by the addition of 0.5 ml of unlabeled methionine (50 mg/ml) and 0.5 ml of potassium cyanide (1 M) before chilling in an ice bath. A total membrane fraction was then prepared by sonication and differential centrifugation (17). Separation of the outer and inner membrane was accomplished by the selective solubilization of the *E. coli* inner membrane by Sarkosyl NL97 (10). Total membrane pellets were resuspended in 5 ml of 0.5% Sarkosyl in distilled water and were incubated for 30 min at 4 C before centrifugation at 100,000 × *g* for 40 min. The Sarkosyl extraction was repeated and the final outer membrane pellet was then resuspended in 100 μl of the sample buffer described by Laemmli (21). After the addition of 10 μl of mercaptoethanol the outer membrane samples were boiled for 2 min immediately before electrophoresis. Volumes (5 μl) of each outer membrane sample were removed before electrophoresis to determine the counts per minute of [^{35}S]methionine in each sample so that an equal number of counts could be applied to each electrophoresis slot to facilitate quantitative comparison. The apparent molecular weight of stained bands on slab gels was estimated by comparison with several standard proteins.

Gel electrophoresis and autoradiography. The discontinuous sodium dodecyl sulfate-polyacrylamide electrophoresis system of Laemmli (21) was used in a slab gel apparatus similar to that described by Ames (1), having a separating gel 18 cm long. For most experiments 13% gels were used to allow good resolution of the region from 10,000 to 80,000 molecular weight. Electrophoresis was initiated at 80 V until the bromophenol blue marker dye had entered the separating gel, at which time the voltage was increased to 160 V. Staining, destaining, and drying of gels was as described by Ames (1). Radioactively labeled gels were contact exposed to Ilford X-ray plates at room temperature. (Under our conditions, 50,000 counts/min of ^{35}S-labeled outer membranes applied to each gel slot required 4 days of exposure to the X-ray film.) After development, the bands on the X-ray film were quantitated on the basis of areas under the peaks of densitometer tracings obtained with a Joyce-Loebl microdensitometer. Occasionally it was necessary to normalize the value of the area under the peak of a particular slot which was unusually narrow, e.g., slot c in Fig. 1. This was achieved by an adjustment relative to a band on the X-ray film that did not change under any experimental conditions used.

In these experiments changes in the incorporation of isotope into outer membrane fractions cannot be ascribed directly to changes in the rate of synthesis of individual proteins, since other factors, such as the rate of translocation from the inner to the outer membrane, could be involved. Therefore, I have limited my description to changes in the relative amount of individual proteins appearing in the outer membrane of *E. coli* during a 3-min pulse with isotope, i.e., changes in the rate of appearance.

Materials. FL1060 (Mecillinam) was a generous gift of Leo Pharmaceutical Products, Ballerup, Denmark. Cephalexin and Sarkosyl were gifts of the Eli Lilly Co. and of Ciba Geigy Chemical Co., respectively. Nalidixic acid was purchased from Sigma Chemical Co. The concentration of nalidixic acid necessary to inhibit DNA synthesis and subsequent septation in *E. coli* B/r was 13 μg/ml.

RESULTS

Outer membrane protein changes during inhibition of normal cell elongation. Inhibition of normal cell elongation in a rod configuration in an exponential culture of *E. coli* B/r by the amidinopenicillanic acid FL1060 was accompanied by changes in the relative amounts of several outer membrane proteins (Fig. 1). Among the more obvious changes were a reduction in the rate of appearance of a protein of 15,000 molecular weight, together with an increase in the rate of appearance of a protein of 80,000 molecular weight. I have called the 15,000-molecular-weight protein "protein G." On a slab gel stained with Coomassie brilliant blue, this protein appears to be the fourth most abundant species in the outer membrane of *E. coli*. The dramatic change in the relative rate of appearance of the 80,000-molecular-weight protein (protein D) together with further details of the unique properties of this protein will be described in full elsewhere (L. J. Gudas and R. James, manuscript in preparation).

The kinetics of the change in rate of appearance of protein G during treatment of an exponential culture of *E. coli* B/r with various concentrations of FL1060 are shown in Fig. 2. Concentrations of FL1060 that were effective in inhibiting the growth of *E. coli* B/r (Fig. 2A) led to a decrease in the rate of appearance of protein G in the outer membrane (Fig. 2B).

Outer membrane protein changes during filament formation. The induction of filaments of *E. coli* B/r by treatment with nalidixic acid was accompanied by changes in the relative rate of appearance of several outer membrane proteins, including the previously described decrease in the Y protein (17); however, the most dramatic change was that of protein G. At concentrations of nalidixic acid that were effective in inhibiting septation of *E. coli* B/r (Fig. 3A), the relative rate of appearance of protein G increased rapidly with the time of treatment (Fig. 3B). With 50 μg of nalidixic acid per ml the relative rate of appearance of protein G increased by some 250% within the first 30 min of treatment. These kinetics are similar to those recently described for the X protein, a cytoplasmic and inner membrane protein of *E. coli* that is also induced by nalidixic acid (11; L. J. Gudas and A. B. Pardee, submitted for publication).

Similar changes in the rate of appearance of protein G were observed during filament formation in *E. coli* MX74 T2 ts27, a *dnaB* mutant, during incubation at the restrictive temperature (Table 1).

The dramatic increase in the rate of appearance of protein G during treatment with nalidixic acid could be the result of de novo synthesis of protein G or of interconversion from other cytoplasmic or membrane protein species. This question was studied in an experiment in which an exponential culture of *E. coli* B/r was pulsed with [^{35}S]methionine for 5 min and then chased in the presence of "cold" methionine, with and without nalidixic acid (Fig. 4). No more labeled protein G was observed in the sample chased in the presence of nalidixic acid as compared with the control sample chased in the absence of nalidixic acid. Since a control culture, pulsed with [^{35}S]methionine after varying times of nalidixic acid treatment, exhibited the characteristic pattern of protein G induction, it is concluded that the protein G that appears in the outer membrane as a consequence of the inhibition of DNA synthesis arises from de novo synthesis.

To determine whether the change in protein G occurring during nalidixic acid treatment or incubation of a *dnaB* mutant at the restrictive temperature was merely a secondary effect associated with filament formation, we studied the outer membrane protein changes occurring after the induction of filaments by cephalexin, a β-lactam antibiotic that appears to be a very specific inhibitor of cell septation in *E. coli*. Although cephalexin at filament-inducing concentrations did induce changes in the rate of appearance of several outer membrane proteins, in particular in protein D, it had no effect on the rate of appearance of protein G (Fig. 5). The same result was obtained if low concentrations of benzylpenicillin were used to induce filamentation.

The results thus far suggest that the rate of appearance of protein G in the outer membrane may be an important determinant of cell shape and cell septation. A reduced rate of appearance of protein G was associated with loss of rod

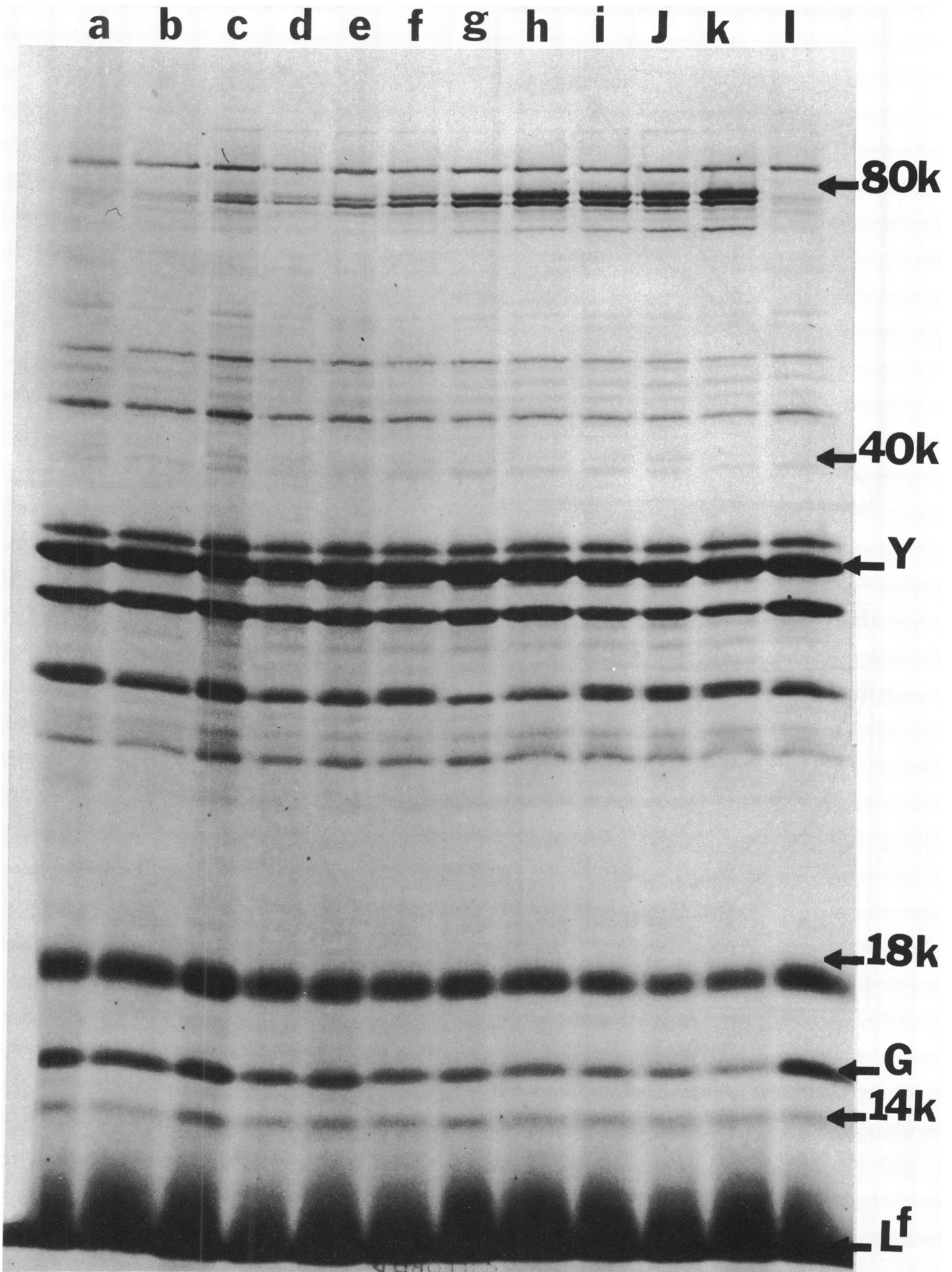

FIG. 1. *Outer membrane protein profiles of an exponential culture of E. coli B/r after treatment with FL1060 (1 μg/ml) for: 0 min (a and l); 10 min (b); 20 min (c); 30 min (d); 40 min (e); 50 min (f); 60 min (g); 70 min (h); 80 min (i); 90 min (j); and 100 min (k). Protein G, protein Y, and the free form of the lipoprotein are indicated, together with the migration of molecular weight standards. The direction of migration is from the top to the bottom of the figure.*

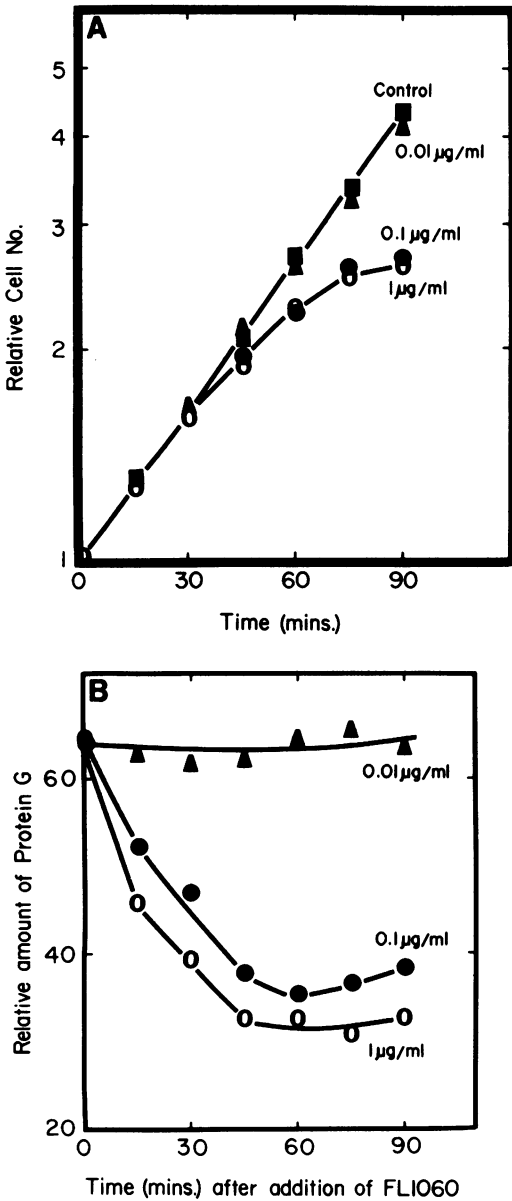

FIG. 2. *(A) Growth of an exponential culture of E. coli B/r after the addition of various concentrations of FL1060 at zero min. (B) Kinetics of the changes in the relative rate of appearance of protein G in the outer membranes of an exponential culture of E. coli B/r after the addition of various concentrations of FL1060 at zero min. The protein G data are expressed as the relative amount of labeled protein G in the outer membrane after a 3-min pulse with [^{35}S]methionine given to part of the culture after the stated time of incubation with FL1060.*

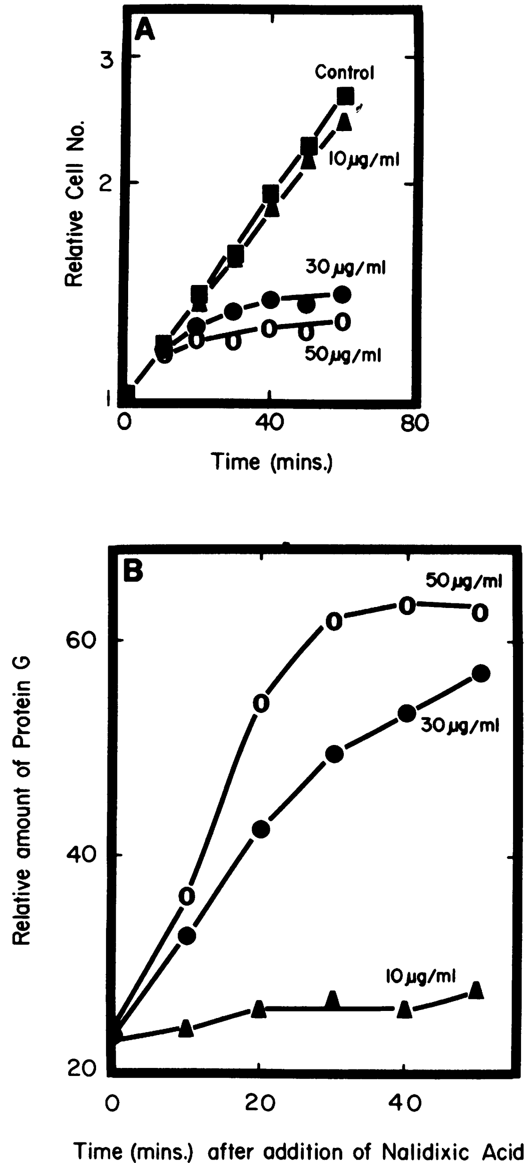

FIG. 3. *(A) Growth of an exponential culture of E. coli B/r after the addition of various concentrations of nalidixic acid at time zero. (B) Kinetics of the changes in the relative rate of appearance of protein G (amount labeled in a 3-min pulse) in the outer membranes of an exponential culture of E. coli B/r after the addition of various concentrations of nalidixic acid at zero min.*

shape during FL1060 treatment, whereas an increased rate of appearance of protein G was associated with filament formation as a consequence of inhibition of DNA synthesis. The absence of any change in the rate of appearance of protein G during filament formation induced by cephalexin or low concentrations of benzylpenicillin suggests that the increased rate of appearance of protein G, found only in specific kinds of filamentous cells, may be a conse-

TABLE 1. *Kinetics of changes in the rate of appearance of protein G in the outer membranes of an exponential culture of E. coli MX74 T2 and E. coli MX74 T2 ts27 (dnaB) during incubation at 42 C*

Time at 42 C (min)	Rate of appearance of protein G	
	MX74 T2	MX74 T2 ts27
0	29	28
10	30	36
20	27	48
30	26	49
40	25	41

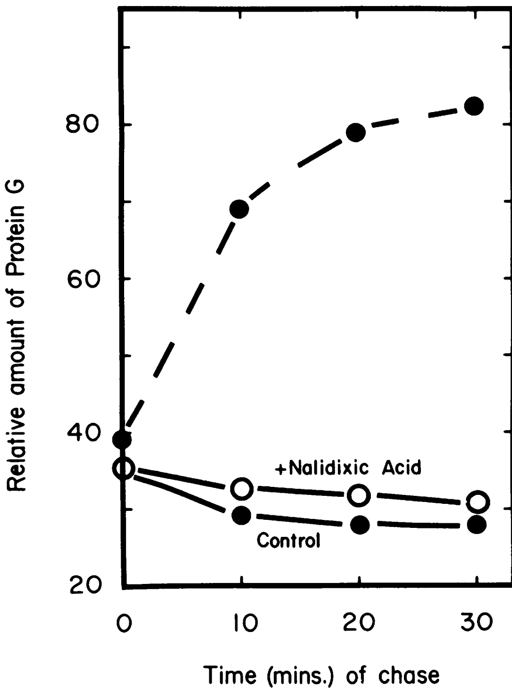

FIG. 4. *De novo synthesis of protein G during nalidixic acid induction. An exponential culture of E. coli B/r was pulsed with [^{35}S]methionine and then chased with* (O) *and without* (●) *50 μg of nalidixic acid per ml. At the times indicated, samples were withdrawn to determine the relative amount of protein G present. The dashed line represents a control culture that was pulsed with [^{35}S]methionine after treatment with nalidixic acid for the times indicated.*

quence of the inhibition of DNA synthesis per se or some closely related event. In an attempt to understand the mechanism of the reduction in the relative amount of protein G during treatment with FL1060, an inhibitor that has no apparent effect on DNA replication itself, we have examined the effect of simultaneous treatment of exponential cultures of *E. coli* B/r with nalidixic acid and FL1060.

Outer membrane protein changes during treatment with FL1060 and nalidixic acid. The effect of nalidixic acid on the rate of appearance of protein G in the outer membrane of *E. coli* B/r cells, after treatment with FL1060 for varying periods of time, is shown in Fig. 6A. Although inhibition of the rate of appearance of protein G by FL1060 did not qualitatively alter the kinetics of the subsequent induction of protein G by nalidixic acid, there was a quantitative reduction in the absolute amount of protein G made in the 30-min assay period. Microscopic analysis revealed that cells exposed to FL1060 for zero or 30 min formed filaments during a subsequent incubation with nalidixic acid, a result in agreement with that of Matsuhashi et al. (22). However, very few cells that had been exposed to FL1060 for 60 min or more formed filaments during incubation with nalidixic acid.

In a similar experiment in which nalidixic acid was added to exponential cultures of *E. coli* B/r after various times of treatment with cephalexin, there was no reduction in the absolute level of protein G by nalidixic acid after 60 min of exposure to cephalexin (Fig. 6B).

The FL1060 effect on the induction of protein G by nalidixic acid was not due to irreversible cell damage or lysis, since even after 2 h of FL1060 treatment full recovery of the capacity for nalidixic acid induction of protein G was seen after the addition of an excess of penicillinase (Fig. 6C).

Preliminary characterization of protein G. During the course of experiments designed to

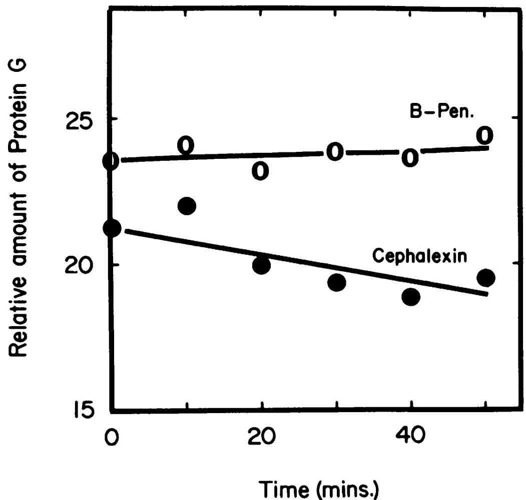

FIG. 5. *Kinetics of the changes in the relative rate of appearance of protein G (amount labeled in a 3-min pulse) in the outer membranes of an exponential culture of E. coli B/r after the addition of cephalexin (4 μg/ml)* (●) *or benzylpenicillin (30 μg/ml)* (O).

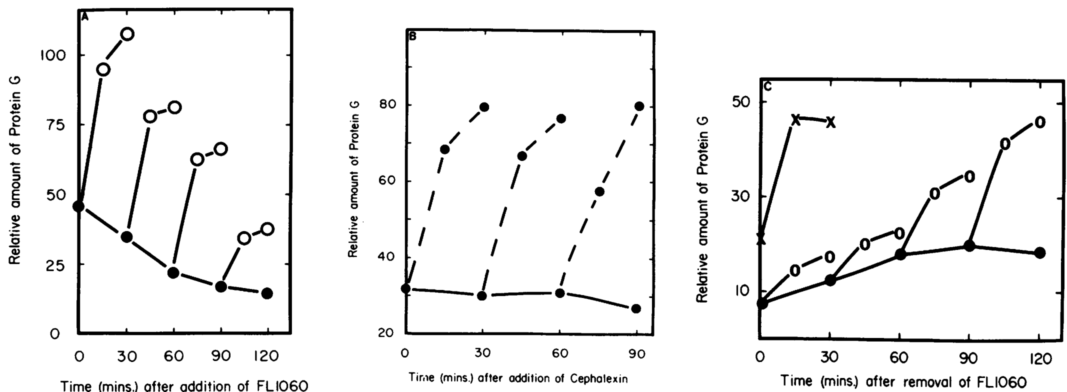

FIG. 6. *Kinetics of the changes in the rate of appearance of protein G (amount labeled in a 3-min pulse) in the outer membranes of an exponential culture of E. coli B/r after the addition of FL1060 at zero min (●). At the times indicated, nalidixic acid (50 μg/ml) was added to part of the culture and incubation was continued for a further 15 or 30 min before pulsing with [35S]methionine to determine the rate of appearance of protein G* (○). *(B) Kinetics of the changes in the rate of appearance of protein G (amount labeled in a 3-min pulse) in the outer membranes of an exponential culture of E. coli B/r after the addition of cephalexin (4 μg/ml) at zero min* (●). *At the times indicated, nalidixic acid (50 μg/ml) was added to part of the culture and incubation was continued for a further 15 or 30 min before pulsing with [35S]methionine* (- - -). *(C) Kinetics of the changes in the rate of appearance of protein G (amount labeled in a 3-min pulse) in the outer membranes of an exponential culture of E. coli B/r that had been incubated with FL1060 (1 μg/ml) for 120 min before the addition of 1,000 U of penicillinase per ml at zero min* (●). *At the times indicated, nalidixic acid (50 μg/ml) was added to part of the culture and incubation was continued for a further 15 or 30 min before pulsing with [35S]methionine* (○). *The changes in the rate of appearance of protein G in a control culture of E. coli B/r after the addition of nalidixic acid alone at zero min were determined* (×).

confirm that a protein band on the gels with an apparent molecular weight of 9,000 represented the free form of the lipoprotein described by Braun and Rehn (5), it became apparent that there was a similarity between the free lipoprotein and protein G. On the basis of continued labeling of the lipoprotein and protein G with [35S]methionine after histidine starvation of *E. coli* B/r *thy his*, these two were the only detectable outer membrane proteins lacking histidine (Fig. 7A–C). This conclusion was supported by the absence of labeled bands in the outer membrane of *E. coli* B/r after labeling with [14C]-histidine (Fig. 7D,E). To determine whether these two proteins are closely related, further similarities were looked for.

One well-described characteristic property of the free lipoprotein is the relative resistance of its biosynthesis to certain antibiotics, such as puromycin and rifampin, but not to chloramphenicol (15). In experiments in which exponential cells of *E. coli* B/r were pulsed with [35S]methionine after a 15-min exposure to puromycin, the only two bands in the outer membrane showing a significant degree of labeling were those corresponding to the free lipoprotein and to protein G (Fig. 7F). The heavy band below the lipoprotein in the puromycin-treated cultures presumably represents prematurely terminated puromycin polypeptide fragments (15); however, there is a definite band present at the position of the lipoprotein and protein G. No significant labeling of any bands was obvious in the cultures incubated with chloramphenicol under the same conditions (Fig. 7G). These results suggest the possibility that the lipoprotein and protein G are directly related to each other.

The absolute molecular weight of 7,200 for the free form of the lipoprotein (4) and the apparent molecular weight of 15,000 for protein G allow for the possibility that protein G is a dimer of the free lipoprotein. This suggestion was investigated by comparing the pattern of labeling of protein G and the lipoprotein with several different 14C-labeled amino acids (Fig. 8A–C). The finding that protein G is labeled with [14C]proline, an amino acid missing in the free form of the lipoprotein (4), together with the very different ratios of [14C]alanine and [35S]methionine incorporated into protein G and the free form of the lipoprotein, indicates that these two proteins do not have identical amino acid compositions.

DISCUSSION

Analysis of the outer membrane protein changes that occur during inhibition of normal

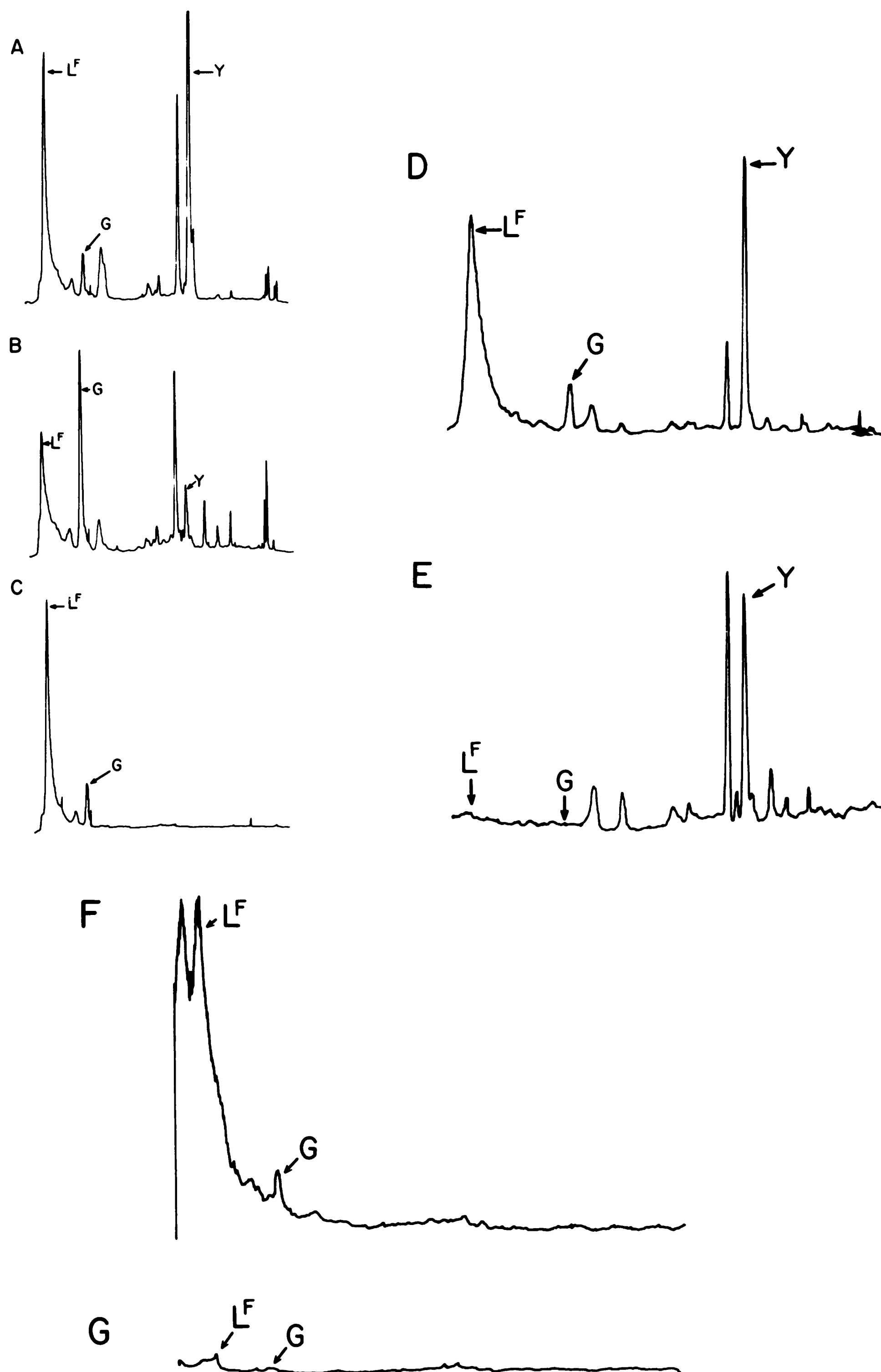

FIG. 7. *Densitometer tracing of an autoradiogram of an outer membrane preparation of an exponential culture of: (A) E. coli B/r labeled with [^{35}S]methionine; (B) E. coli B/r labeled with [^{35}S]methionine after a 15-min incubation with nalidixic acid (50 μg/ml); (C) E. coli B/r thy his labeled with [^{35}S]methionine after a 60-min starvation for histidine; (D) E. coli B/r labeled with [^{35}S]methionine; (E) E. coli B/r labeled with [^{14}C]histidine; (F) E. coli B/r labeled with [^{35}S]methionine after a 15-min incubation with puromycin (800 μg/ml); (G) E. coli B/r labeled with [^{35}S]methionine after a 15-min incubation with chloramphenicol (100 μg/ml). Protein G, protein Y, and the free lipoprotein are indicated.*

cell elongation (by FL1060) and during abnormal cell elongation (in filaments induced upon inhibition of DNA synthesis) has singled out several proteins that may have important roles in cell division of *E. coli*. The decrease in the rate of appearance of protein G during FL1060 treatment, together with the rapid increase in its rate of appearance during nalidixic acid-induced filament formation, suggests a possible important role for this protein in cell elongation.

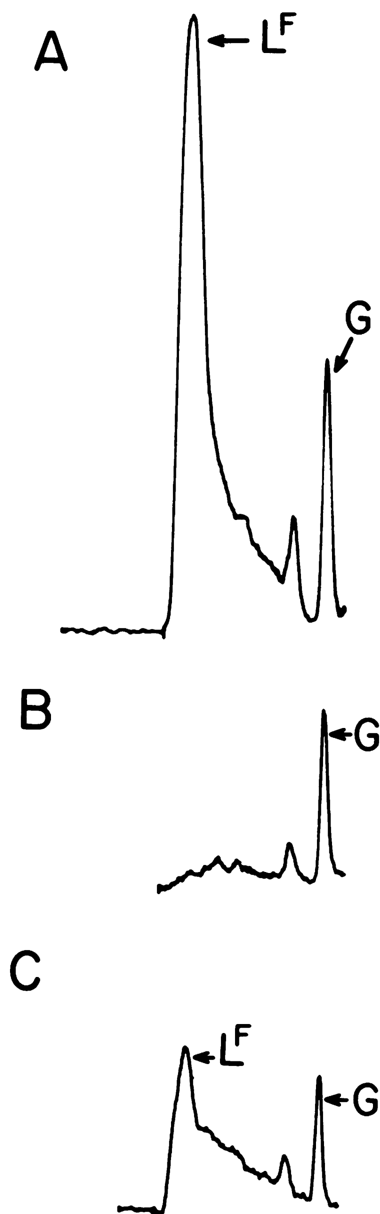

FIG. 8. *Densitometer tracing of a part of an autoradiogram of an outer membrane preparation of an exponential culture of E. coli B/r labeled with: (A) [^{35}S]methionine; (B) [^{14}C]proline; (C) [^{14}C]alanine. Protein G and the free form of lipoprotein are indicated.*

The observation that protein G is induced as a consequence of treatments that cause filamentation by inhibition of DNA synthesis, but not as a consequence of treatment with filament-inducing concentrations of cephalexin or benzylpenicillin, indicates that the increased rate of appearance of protein G in the outer membrane is the result of inhibition of DNA synthesis, or a closely related event, and is not just a nonspecific secondary alteration associated with filament formation. This biochemical difference between nalidixic acid and cephalexin- or benzylpenicillin-induced filaments, i.e., differences in the rate of appearance of protein G, may explain the obvious differences between these types of filaments as indicated by treatment with a high concentration of benzylpenicillin as a probe of presumptive septum sites (26).

The apparent differences in the kinetics of the changes in rate of appearance of protein G with increasing concentrations of nalidixic acid may in fact be an artifact (Fig. 3B). The variability in resistance of the biosynthesis of individual outer membrane proteins to inhibitors of protein synthesis is well documented (15). Since nalidixic acid, at concentrations above those necessary to inhibit DNA synthesis in *E. coli* B/r, also results in progressively greater inhibition of protein synthesis (R. James, unpublished data), it is conceivable that measurements of one particular protein, as a fraction of a constant number of counts per minute of outer membrane preparation applied to each gel slot, could be misleading. However, the likely errors due to this phenomenon are not sufficiently large to alter the general conclusions derived from these experiments. One should perhaps worry more when measuring the rate of appearance of a more minor outer membrane component under these same conditions.

The de novo nature of the induction of protein G by nalidixic acid suggests that protein G may be the factor, or a factor, in filament formation, one of the SOS functions which are inducible as a consequence of the inhibition of DNA synthesis (24). This conclusion is supported by analysis of the rate of appearance of protein G in *tif* mutants of *E. coli* B/r (30) and in revertants of *tif* mutants in which the SOS functions are thermally inducible at the restrictive temperature without an effect on DNA synthesis (R.

James and A. Brenner, manuscript in preparation).

The proposed relationship between the rate of appearance of protein G in the outer membrane and the expression of cell envelope elongation in *E. coli* B/r is supported further by experiments in which cells of an exponential culture of *E. coli* B/r were treated with FL1060 for varying periods of time and then were exposed to nalidixic acid for 15 to 30 min before pulsing with [^{35}S]methionine to determine the rate of appearance of protein G. The longer the treatment with FL1060, the smaller was the absolute increase in the rate of appearance of protein G induced by nalidixic acid. Cells that had been exposed to FL1060 for 30 min or less formed filaments during the subsequent nalidixic acid treatment. However, cells exposed to FL1060 for longer than 30 min formed a high percentage of large spherical cells during exposure to nalidixic acid.

As a result of this work, FL1060 and cephalexin, two β-lactam antibiotics that have a different timing of action on *E. coli* and whose action results in different morphological effects, can now be distinguished biochemically on the basis of their effect on the rate of appearance of protein G in the outer membrane of *E. coli* B/r. This is the first recorded biochemical difference between the action of these two very chemically similar compounds and may be a helpful clue in resolving their mechanism of action, especially since the specific inner membrane binding proteins for each of these compounds have recently been resolved (27, 28).

It must be assumed at this stage that the FL1060- and cephalexin-sensitive biochemical events are involved in the biosynthesis or modification of murein and that it is a change in the murein configuration during FL1060 treatment that triggers the decrease in the rate of appearance of protein G. A precedent for the situation in which an alteration in murein could affect an outer membrane protein already exists in *E. coli*, since the free form of the lipoprotein is covalently cross-linked to murein after both components are inserted in place (3). Thus a reduction in the diaminopimelic acid acceptor sites on the murein pentapeptide chain for cross-linking of the lipoprotein (6) (presumably by a transpeptidase-type reaction) could affect the incorporation of the lipoprotein into the outer membrane. The possibility of a similar situation existing for protein G is perhaps suggested by our finding of a similarity between protein G and the free form of the lipoprotein.

The close similarity between protein G and the free form of the lipoprotein, in that they are the only two outer membrane proteins lacking histidine and whose biosynthesis is relatively resistant to puromycin but not to chloramphenicol, is striking. It has not yet been conclusively demonstrated that the resistance of the biosynthesis of the lipoprotein to puromycin is the result of an intrinsic property of the messenger ribonucleic acid for this protein, but the data thus far do suggest that there is something unique in the biosynthesis of this protein (25; S. Halegova, A. Hirashima, and M. Inouye, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K123, p. 167) that is presumably shared by protein G.

Because of the observed differences in labeling patterns with various ^{14}C-labeled amino acids, it is unlikely that protein G is a simple dimer of the free lipoprotein; indeed, the results of the pulse-labeling experiment (Fig. 3) demonstrate that that free lipoprotein, once it is inserted in the membrane, cannot be a precursor of protein G. At the present time the possibility that protein G is a precursor of the free lipoprotein has not been completely eliminated; however, such a conversion would presumably involve cleavage and an asymmetric distribution of the amino acid content of protein G into two or more such units. Hopefully the isolation and detailed characterization of protein G will answer many of these question.

A working hypothesis to explain the data is that cell elongation and cell septation in *E. coli* are the result of two transient mechanisms that provide spatial information to the growing murein sacculus. Combination of newly formed murein with specific outer membrane proteins, including protein G, could determine normal cell elongation. The elongation process is assumed to initiate at a specific time in the cell cycle under the influence of a special murein structure, the formation of which requires the activity of penicillin-binding protein 2, which is FL1060 sensitive (28). Inhibition of the initiation of new rounds of elongation by FL1060 would thus prevent the normal insertion of protein G into the outer membrane, except that occurring in already initiated rounds of cell elongation that are known to be resistant to FL1060 (20). Thus protein G would fulfill the function of a cell elongation structural protein.

Exposure of an exponential culture of *E. coli* B/r to FL1060 would progressively reduce the percentage of cells in which cycles of cell elongation and protein G insertion were in progress and thus would quantitatively reduce the subsequent level of induction of protein G by nalidixic acid. This model would provide an explanation for the inability of spherical FL1060-

resistant mutants of *E. coli* K-12 to filament in the presence of nalidixic acid (22). We are examining similar mutants to determine whether they have a lower rate of appearance of protein G in their outer membranes and a lower absolute level of inducibility of protein G by nalidixic acid.

By analogy, septation could depend upon combination of a different set of proteins, of which protein G is not normally a major component, with newly formed murein. The initiation event for septation presumably depends upon a murein structure that requires the transient activity of penicillin-binding protein 3 (27). An above-normal rate of appearance of protein G in the outer membrane is presumed to be involved in inhibition of septation, perhaps by a saturation process in which potential septum sites are temporarily inactivated. In this context protein G, which when present at a normal rate of appearance coordinates normal cell elongation as a rod with septation, would be acting as a negative controller of cell division. Such a function has often been proposed (7, 9, 12, 19, 29), presumably as a protective device to prevent cell death that would result from premature cell division before termination of DNA replication. Of course at this moment the increased rate of appearance of protein G occurring during filamentation as a consequence of DNA synthesis inhibition can only be regarded as a correlation. In a subsequent paper I hope to further develop this correlation.

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