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Effect of Growth Medium on the Relative Polypeptide Composition of Cellular Outer Membrane and Released Outer Membrane Material in *Escherichia coli*

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When ratios of the major polypeptides of the outer membrane isolated from cells of *Escherichia coli* B grown in minimal medium containing either a single amino acid or several amino acids were compared, no difference was observed. However, the ratio of these polypeptides in outer membrane material released into the medium during logarithmic phase growth on these two media was markedly different.

Escherichia coli releases outer membrane material into the medium during log phase growth as well as during the stasis associated with stationary phase or with inhibition of protein synthesis (6, 13). Release also occurs immediately upon adsorption of T4 phage to the cell surface, when up to 30% of the total outer membrane can be released within a few minutes (8). We have studied the composition of this released material to understand its structural and functional relationship to cellular outer membrane.

The *E. coli* B substrain used in our experiments has three major polypeptides above 20,000 daltons in its outer membrane, II* (33,000 daltons), Ia (36,000 daltons), and a (37,000 daltons). (The nomenclature for outer membrane proteins II* and Ia is that of Schmitges and Henning [15]. For an explanation of the identification of II*, Ia, and a in *E. coli* B, see Loeb and Kilner [8a]). II* is the predominant polypeptide, the ratio of II* to Ia + a in outer membrane, prepared by a variety of procedures, being about 2.0 (8a). (Note: Since proteins Ia and a are not sufficiently resolved upon gel electrophoresis, the data are presented as the sum Ia + a.)

Previously we found that, when *E. coli* B was grown in M9-Casamino Acids, the outer membrane material released into the medium during log phase growth or upon infection with T4 phage was markedly deficient in II*, the ratio of II* to Ia + a being about 0.5 (8a). Hence, the released outer membrane material was not representative of the outer membrane as a whole. However, a study from another laboratory did not find a deficiency of II* in outer membrane material released from two *E. coli* K-12 strains in stationary phase under limiting oxygen conditions (5). The difference in results from these two laboratories could be due to strain differences, to the considerable differences in growth conditions of the bacteria, or to the different methods of isolation of released outer membrane.

In this communication we describe an effect of the growth medium on the polypeptide composition of outer membrane material released from *E. coli* B cells during log phase growth. Two kinds of media were used: (i) M9-Casamino Acids (1 mg/ml) medium with either radioactive leucine or radioactive arginine added to label the cells, and (ii) M9-single-amino-acid medium, the single amino acid being radioactive leucine or arginine (0.1 μmol/ml). After five generations of growth, both outer membrane and released outer membrane were obtained and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the ratios of radioactivity in II* to radioactivity in Ia + a were determined.

Figures 1A and B show, in an experiment in which [^{3}H]leucine was used to label the cells, that whether cells are grown in M9-Casamino Acids (Fig. 1A) or in M9 containing only the single amino acid, leucine (Fig. 1B), the ratio of II* to Ia + a in outer membrane is essentially the same, namely 2.10 and 1.80, respectively. This is not true, however, for released outer membrane in which the ratio of II* to Ia + a is 0.50 for cells grown in M9-Casamino Acids and 1.20 for cells grown in M9-single amino acid. Thus, growth conditions which do not grossly alter the protein composition of the outer membrane do affect the protein composition of that

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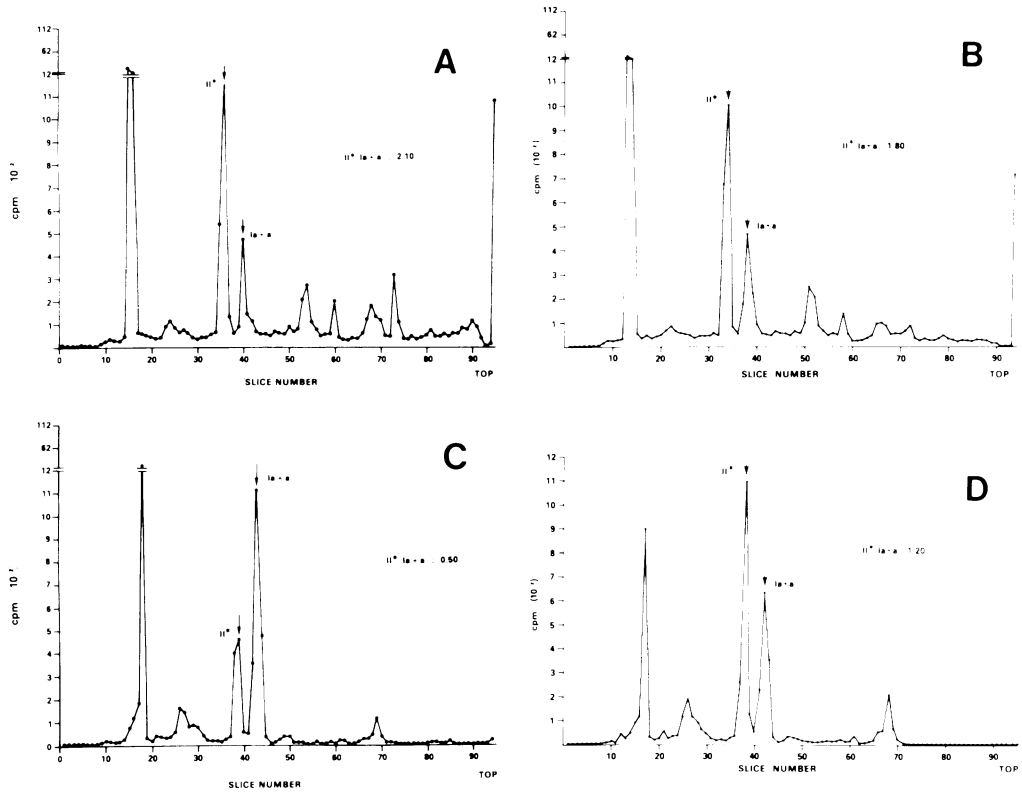

FIG. 1. *Protein composition of outer membrane and released outer membrane obtained from cells grown in M9-Casamino Acids and M9-leucine. A 7-ml volume of E. coli B was grown to 5 × 10⁷ cells per ml in M9-Casamino Acids or M9-leucine. The former consisted of M9 salts (1), 1.3 mg of glucose per ml, 1 mg of Casamino Acids (Difco) per ml, and L-[4,5-³H]leucine (70 μCi/ml; 53 Ci/mmol). M9-leucine medium was similar except that it contained 0.1 μmol of L-[4,5-³H]leucine (70 μCi/ml) per ml instead of the Casamino Acids. The cultures were chilled and separated by centrifugation (8a) into cell pellet and supernatant fluid containing the released material. Outer membrane was derived from the former after first combining it with an unlabeled cell pellet obtained from cells grown in 200 ml of comparable medium. After breakage of the cells by sonic treatment, total membrane was obtained by differential centrifugation, and outer membrane was obtained from total membrane after extraction with Triton X-100 (8a, 15). To obtain the released outer membrane, the total released material was dialyzed and then centrifuged through a sucrose gradient to yield the outer membrane fraction; this was dialyzed to remove sucrose and lyophilized. These procedures have been described in detail previously (8a). Samples of outer membrane and released outer membrane were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the Laemmli system (7, 8a); after electrophoresis, the gels were frozen, sliced, and counted. (A and B) Outer membrane from cells grown in M9-Casamino Acids and M9-leucine, respectively. (C and D) Released outer membrane from cells grown in M9-Casamino Acids and M9-leucine, respectively.*

part of the outer membrane which is released.

In the above experiment leucine was used as the single amino acid. Leucine, however, is toxic for *E. coli* (11, 12), and, in fact, the growth rate of our cells is slower in M9-leucine (division time, 54 min) than in M9 with no additions (division time, 50 min). Therefore, to ascertain that the above result is not peculiar to leucine, an experiment with another amino acid was performed. Cells were grown in each of the following media: (i) M9-Casamino Acids medium with L-[*U*-¹⁴C]leucine added to label the cells, (ii) M9-leucine medium with L-[*U*-¹⁴C]leucine added to label the cells, (iii) M9-Casamino Acids medium with L-[*U*-¹⁴C]arginine added to label the cells, and (iv) M9-arginine medium with L-[*U*-¹⁴C]arginine added to label the cells. Outer membranes were obtained from the cells, total released material was obtained from the medium, and the ratios of II* to Ia + a in each of

these were determined after electrophoresis. Note that in this experiment that total released material rather than the outer membrane fraction of released material was used. This simplification was permissible because control experiments had established that the ratio of II* to Ia + a is the same in both types of preparations. The results appear in Table 1.

It is again apparent that these particular growth media do not affect the relative amounts of proteins II* and Ia + a in the outer membrane. Thus, ratios of 1.90 and 1.80 were found in the outer membrane from leucine-labeled cells grown in M9-Casamino Acids and M9-leucine, respectively; and similarly, ratios of 2.30 and 2.50 were obtained for outer membrane from arginine-labeled cells grown in M9-Casamino Acids and M9-arginine, respectively. Although the [^{14}C]arginine-labeled cells had a higher ratio of II* to Ia + a, this is probably a reflection of the amino acid composition of these proteins.

However, it is also apparent that the medium affects the composition of released outer membrane. Thus [^{14}C]leucine-labeled cells grown in M9-Casamino Acids medium released material with a ratio of II* to Ia + a of 0.60, whereas such cells grown in M9-leucine released material with a ratio of II* to Ia + a of 1.30. Similarly, material released from [^{14}C]arginine-labeled cells grown in M9-Casamino Acids medium had a ratio of II* to Ia + a of 0.40, whereas that released from cells grown in M9-arginine medium had a ratio of II* to Ia + a of 0.70. Hence, regardless of the amino acid present in M9-single-amino-acid medium, the outer membrane released is richer in II* than when M9-Casamino Acids medium is used. This indicates that factors other than leucine toxicity are responsible for the results when leucine was the single amino acid in M9-single-amino-acid medium.

The above experiments indicate that conditions which do not alter the relative proportions of proteins II*, Ia, and a in the outer membrane do, however, affect their distribution in the outer membrane, as reflected in the varying proportions of these proteins in that fraction of the outer membrane which is released. It is interesting to note in this regard that visual evidence of an altered organization of the outer membrane has been shown in electron micrographs of septum formation in *E. coli* B/r, in that cells grown in minimal medium containing glucose and Casamino Acids often form septa in a different manner than cells grown in minimal medium containing only glucose (3). However, the reasons for this altered organization are purely conjectural at this point. Perhaps the increased amount of amino acid transport occurring in cells growing in medium containing several amino acids as opposed to a single amino acid is responsible. This is feasible because the outer membrane and some of its proteins in particular have been shown to play a key role in the passive transport of these molecules (2, 4, 9, 10). It is also possible that our results are due to the different metabolic environments of the cells in each of the different media or to the type of outer membrane organization needed to enable the faster growth rate observed in the richer medium.

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TABLE 1. *Effect of media on the relative amounts of II* and Ia + a in outer membrane and total released material*[a]

Composition of medium	Source of label	Outer membrane			Total released material		
		II* (cpm)	Ia + a (cpm)	Ratio of II* to Ia + a	II* (cpm)	Ia + a (cpm)	Ratio of II* to Ia + a
Casamino Acids	[^{14}C]leucine	730	390	1.90	640	1,110	0.60
Leucine	[^{14}C]leucine	1,260	690	1.80	1,990	1,560	1.30
Casamino Acids	[^{14}C]arginine	670	290	2.30	740	2,090	0.40
Arginine	[^{14}C]arginine	960	380	2.50	910	1,270	0.70

[a] A 7-ml volume of cells was grown in M9 salts containing 1.3 mg of glucose per ml with the following additions to the media: (i) 1 mg of Casamino Acids per ml plus L-[*U*-^{14}C]leucine (8 μCi/ml; 348 mCi/mmol); (ii) 0.1 μmol of L-[*U*-^{14}C]leucine (8 μCi/ml) per ml; (iii) 1 mg of Casamino Acids per ml plus L-[*U*-^{14}C]arginine monohydrochloride (8 μCi/ml; 324 mCi/mmol); or (iv) 0.1 μmol of L-[*U*-^{14}C]arginine monohydrochloride (8 μCi/ml) per ml. Outer membrane was obtained as described in the legend to Fig. 1. Total released material was obtained from the supernatant fluid by precipitation with cold trichloroacetic acid in the presence of RNA as carrier, as previously described (8a). All samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the distribution of radioactivity across the gel was determined as described in the legend to Fig. 1.

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