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Membrane Protein Biosynthesis in Bacteriophage BF23-Infected *Escherichia coli*

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When *Escherichia coli* is infected with bacteriophage BF23, two new proteins with molecular weights greater than 10,000, as indicated by polyacrylamide gel electrophoresis, are found associated with the cells' membranes. One of these, found associated with both the inner and outer membrane, has a molecular weight of about 55,000 and is regulated by the A1 gene of this phage, a gene found on the spontaneously injected 8% piece of BF23 DNA, DNA that codes for the synthesis of proteins necessary for the injection of the whole phage genome. The other protein, often undetected in whole membrane preparations, is found exclusively associated with the inner membrane. Evidence indicates that this protein is also regulated by the initially injected 8% piece of the DNA.

Bacteriophage BF23 is a very close relative of T5 (13). The physiology of infection by these phages is unique in that the transfer of the phage DNA molecule occurs in two steps. When these phage attach to their host, *Escherichia coli*, only 8% of the DNA molecule is spontaneously injected. This 8% of the DNA, the first-step-transfer DNA, must induce the synthesis of new proteins before the remaining 92% of the DNA can enter the cell (11, 13). The "pre-early" proteins coded for by this first-step-transfer DNA and synthesized from 1 min postinfection to about 10 min postinfection (2, 13-15), besides controlling the transfer of the whole phage genome, are responsible for the shut-off of host macromolecular synthesis, the shut-off of "pre-early" protein synthesis, and the abortive infection that occurs in the presence of the colicinogenic factor Col Ib (11, 13, 15, 17, 18). It has been shown, by the use of short pulses of radioactive amino acids and mutants that are unable to transfer the whole DNA molecule, that at least four polypeptides are synthesized during this pre-early phase of infection (14, 15).

We have found that one of the proteins induced by the first-step-transfer DNA of T5 is a major protein of the cell envelope of the infected cells. Polyacrylamide gel electrophoresis of the proteins solubilized from the cells' membranes indicates that this protein can account for as much as 15% of the newly synthesized membrane-associated protein in T5-infected *E. coli* B (6). We have found that amber mutants in the A1 gene of T5 that cannot transfer the whole DNA molecule to the cell (2) do not induce the synthesis of this protein, but phage that are mutant in the A2 gene and also cannot transfer the whole DNA molecule (2) do induce its synthesis (7). We concluded that the A1 gene may be the structural gene for the T5-induced major membrane protein.

To see if we could confirm the fact that one of the T5 pre-early genes coded for a major membrane protein, we have studied membrane protein biosynthesis after infection of *E. coli* by the related phage, BF23. We have found two new membrane proteins after BF23 infection, one of them analogous to the major membrane protein of T5-infected cells and missing in A1$^-$ mutants of BF23 and another of lower molecular weight, often undetectable in whole membrane fractions, that is found exclusively associated with the inner membrane. Studies of the inner membrane of T5-infected cells indicate that this protein is also induced by T5.

MATERIALS AND METHODS

Organisms and media. *E. coli* B was obtained from the laboratory of M. J. Bessman and has been subcultured in our lab since 1968. At the beginning of this study, we found it to be resistant to BF23. *E. coli* B/5 was obtained from R. Benzinger; it is sensitive to BF23. *E. coli* JK20 (*E. coli* W3110 F$^-$, *str*^{r}, λ$^-$ [Col Ib-P9]) (a K-12 strain) was obtained from J. Konisky, and *E. coli* CR63 (permissive for amber mutants) was obtained from M. L. Dirksen. T5 phage, BF23 phage, and BF23*am*57 (A1$^-$) phage were obtained from R. Benzinger. M-9 medium was made as described by Herriott and Barlow (8). Phage stocks were grown in the appropriate hosts by the confluent lysis method (1). All liquid cultures were aerated by shaking.

Isolation of bacterial membranes. In most of our

experiments, a double-labeling procedure was used in which proteins made in uninfected cells were labeled with ^{14}C-amino acids, and proteins made in infected cells were labeled with ^{3}H-amino acids. The two cultures were mixed before the cells were collected, and the membranes were isolated from the mixture.

Cells were grown in M-9 medium containing 0.5% glucose, 0.05% yeast extract, 5×10^{-4} M $CaCl_2$, and 10 μg each of leucine and tyrosine per ml from a 5% overnight inoculum in the same medium. Cultures of 100 ml were routinely used. Infection was carried out when the cells reached a concentration of about 8.5×10^{8} cells/ml, using a multiplicity of 10 phage/cell. More than 99% of the cells were infected this way. [^{3}H]leucine and [^{3}H]tyrosine were added at the indicated times after infection. Uninfected cells that had been labeled with [^{14}C]leucine and [^{14}C]tyrosine were chilled on ice, and 100 μg of chloramphenicol was added per ml of culture. Amino acid incorporation in the infected cells was stopped by mixing with the chilled, uninfected culture containing chloramphenicol. The cells from the mixture of infected and uninfected cells (usually 150 ml) were removed by centrifugation, washed once with 30 to 40 ml of 0.01 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 7.4, and resuspended in 15 ml of HEPES. Washing and resuspension of the cells could also be done with 0.05 M Tris, pH 7.8. The cells were broken by one or two passages through a French pressure cell. $MgCl_2$ was added to give a final concentration of 0.01 M, and about 1 mg each of DNase and RNase was added. The unbroken cells were removed by centrifugation at 5,000 rpm, and the cell envelope was collected by centrifugation for 60 min at 50,000 rpm in a 50 (Spinco) rotor at 4°C and washed with 7 ml of HEPES.

Triton extraction. For Triton extraction the washed pellet was resuspended in 6.3 ml of HEPES. A 0.7-ml portion of 20% Triton X-100 was added, and the mixture was incubated at 22 to 25°C for 20 min. The Triton-insoluble (outer membrane [22]) fraction was removed by centrifugation for 60 min at 50,000 rpm. The Triton-soluble (inner membrane [22]) proteins were precipitated by the addition of 2 volumes of 95% ethanol to the Triton supernatant solution, followed by storage overnight in the freezer. The proteins were then collected by a short centrifugation at 8,000 rpm.

Sucrose gradient centrifugation. The mixtures of infected and uninfected cells grown and infected and labeled as described above were collected and resuspended in 150 ml of distilled water. These were incubated at 37°C for 30 min and then recentrifuged. This step was found to be essential for good separation by gradient centrifugation of inner and outer membranes of *E. coli* B. After the incubation in distilled water, the cells were centrifuged and resuspended in either HEPES buffer (pH 7.4, containing 1 mM EDTA) or 0.01 M Tris buffer (pH 7.8, containing 1 mM EDTA) for washing and cell breakage. Both buffers gave comparable results. The membrane pellet collected by centrifugation for 60 min at 50,000 rpm was washed with 7 ml of HEPES and resuspended in 1.5 ml of HEPES. A 1.0-ml amount of this membrane suspension was layered on a 30 to 50% sucrose step gradient. The remaining 0.5 ml was used for solubilization and electrophoresis of membrane proteins. The gradient was made up of solutions of sucrose dissolved in HEPES (from the bottom): 5 ml of 50% sucrose, 10 ml of 45% sucrose, 5 ml of 40% sucrose, 5 ml of 35% sucrose, and 5 ml of 30% sucrose, all over a 1-ml cushion of 65% sucrose. The percentage of sucrose was checked by measurement of the refractive index on a Bausch & Lomb refractometer. After layering the membrane suspension on the top, the gradient was centrifuged at 25,000 rpm for 18 h in an SW25.1 (Spinco) rotor at 4°C. Two bands could be visualized after the centrifugation. The gradient was removed from the top to bottom by perfusion of 65% sucrose into the bottom of the tube and collected in a Gilson (FC 80-H) microfractionator in 0.5-ml fractions. Density, radioactivity, and optical density at 280 nm were determined for each fraction. Fractions containing the bands (8 through 18 and 49 through 55) were combined. They were found to have densities of 1.15 and 1.22, respectively. These peak fractions were diluted with 10 volumes of HEPES and centrifuged at 50,000 rpm for 2 h to collect the inner (lighter) and outer (heavier) membranes.

Preparation of samples for gels. Samples were prepared for electrophoresis by the method of Schnaitman (23). The whole membrane pellet, Triton-insoluble pellet, ethanol precipitate, or inner or outer membrane from the sucrose gradient was suspended in 0.1 M phosphate buffer, pH 7.2, containing 0.6% sodium dodecyl sulfate, 4×10^{-3} M EDTA, and 0.08% mercaptoethanol at a concentration of about 7 mg of protein per ml (as assayed by the method of Lowry et al. [12]).

The tubes containing the sample in this solution were capped after having the air replaced by N_2 (as recommended by Schnaitman [21]) and incubated at 37°C for 2 h. The samples were then dialyzed overnight at room temperature against 50 to 100 volumes of a solution of 0.1 M sodium phosphate buffer, pH 7.2, containing 8 M urea, 5×10^{-4} M EDTA, 0.1% sodium dodecyl sulfate, and 0.1% 2-mercaptoethanol. This solution must be prepared fresh before use. Dialysis was done in screw-capped bottles in which the air had been replaced by N_2. After the overnight dialysis, the samples were boiled for 5 min, a 1:25 dilution of 1% bromophenol blue was added, and they were then ready for electrophoresis. They may be frozen and stored at this point. We routinely subject them to electrophoresis within 2 days, however.

Gel electrophoresis. Gels were prepared according to the procedure of Maizel (16). A 3.75-g portion of acrylamide, 1.5 g of urea, and 0.10 g of bisacrylamide were dissolved in 50 ml of gel buffer (0.1% sodium dodecyl sulfate in 0.1 M sodium phosphate buffer, pH 7.2) at room temperature, and 10 mg of $(NH_4)_2S_2O_8$ was added. After this was dissolved, 30 μl of *N,N,N',N'*-tetramethylethylenediamine (TEMED) was added, and the solution was degassed with a vacuum pump and put into gel tubes (15 by 0.5 cm) that had been precoated with Canalco Column-coat and dried. The acrylamide solution was

overlaid with distilled water. After polymerization (30 to 60 min), the gels were left undisturbed for about 18 h. The water was then removed, and the tubes were filled with the gel buffer to be placed in the upper chamber. The sample containing about 50 μg of protein was then injected onto the top of the gel with a Hamilton syringe, and the gels were run at 5 mA/gel for 9 to 10 h. In the Maizel gel system, the upper and lower buffers consisted of the gel buffer described above. In the Bragg-Hou gel system, the upper buffer consisted of 0.1 M sodium phosphate buffer (pH 11.4) and the lower buffer consisted of 0.1 M sodium phosphate buffer (pH 4.1), with both buffers containing 0.1% sodium dodecyl sulfate. Unless otherwise indicated, the Bragg-Hou system was used. After the electrophoresis was complete, the gels were removed from the tubes and frozen or stained with Coomassie blue. After 6 h, the stained gels were destained with several changes of destaining solution consisting of 25% methanol and 13% glacial acetic acid. Gels to be analyzed for radioactivity were barely thawed and sliced with a cheese slicer type of gel slicer made by Earl Sandbeck at Johns Hopkins School of Medicine. Approximately 80 slices/gel were obtained. The slices were placed in scintillation vials containing 0.5 ml of a solution containing 9 parts NCS solubilizer and 1 part water. The vials were heated (tipped so that each slice is covered with NCS solution) at 50°C for 2 h. A 10-ml portion of toluene-2,5-diphenyloxazole1,4-bis-(5-phenyloxazolyl)benzene counting fluid was added and the samples were counted in a Beckman LS230 scintillation counter.

Analysis of results. Results from double-label membrane protein experiments were routinely analyzed by computer using a program developed by George Policello of the Biostatistics Department at the University of Florida. The counts are corrected for background and "spill," and then the actual counts per minute per slice are plotted versus the slice number. The percentage of the total ^3H or ^{14}C in each slice is calculated and plotted, as is the difference ($^3H - ^{14}C$) in these percentages.

RESULTS

Membrane protein profiles of uninfected and BF23-infected *E. coli*. Figure 1a and b shows the results of an experiment in which *E. coli* B/5 was infected at 0 time with phage BF23 and then pulse labeled for 12 min with tritiated amino acids. The cells were then chilled and mixed with a culture of *E. coli* B/5 that had been labeled for 2 h with ^{14}C-amino acids. The membranes were isolated from these cells, and the proteins were solubilized and subjected to electrophoresis with the Bragg-Hou system. Figure 1a shows the percentage of total ^3H and ^{14}C in each slice, and Fig. 1b shows the difference in these percentages. Positive differences are indicative of proteins made in infected cells but not in uninfected cells, and negative differences are indicative of proteins made in uninfected cells but not in infected cells. It can be seen that there are substantial amounts of two phage-induced proteins. Peak I is analogous to the major membrane protein (MMP) of T5-infected cells. Peak II is a smaller protein often not visible in the whole membrane fraction due to its proximity to the major outer membrane proteins of *E. coli*. The Bragg-Hou gel system usually provides enough separation of these proteins so that phage protein II may be seen; using the Maizel system, it is rarely seen in the whole membrane fraction, however. Figure 1c is a control experiment in which cells were not infected but were pulse labeled for 12 min with ^3H-amino acids before mixing them with a culture that had been labeled for its entire growth period with ^{14}C-amino acids. The difference seen at the lower end of the gel in Fig. 1a and b represents material of below about 10,000 molecular weight, since it cannot be resolved even on 15% gels.

When *E. coli* JK20 (an *E. coli* K-12 strain containing a Col Ib plasmid) was infected with BF23 and pulse labeled for 0 to 7 min postinfection, phage protein II was completely obscured by its proximity to host outer membrane protein 3 (Schnaitman's [24] terminology), the proportion of protein 3 being greater in this strain than in our *E. coli* B. The same pattern is seen if the cells are labeled from 7 to 14 min postinfection. When the whole membrane is separated into its inner and outer components, however, phage protein II is plainly visible (see below). Protein I is much more difficult to see in T5-infected *E. coli* K-12 strains than in B strains (6, 7). The BF23 protein I appears in approximately equal amounts in these two strains, however.

Separation of the inner and outer membranes in BF23-infected cells. To determine whether the phage-induced proteins were present in the inner or outer membranes of these cells, the whole membrane fraction was extracted with Triton X-100 in the presence of Mg^{2+}. This treatment solubilizes proteins of the inner membrane (22). The proteins are collected by precipitation with alcohol and prepared for electrophoresis as indicated in Materials and Methods. The Triton-insoluble fraction is also prepared as indicated. Electrophoresis was with the Bragg-Hou system. The proteins found in the Triton-insoluble, presumably outer membrane fraction of BF23-infected *E. coli* B/5 are indicated in Fig. 2a and b. The Triton-soluble proteins are shown in Fig. 2c and d. The results show phage proteins I and II in the inner membrane. A small amount of protein I is found also in the outer membrane

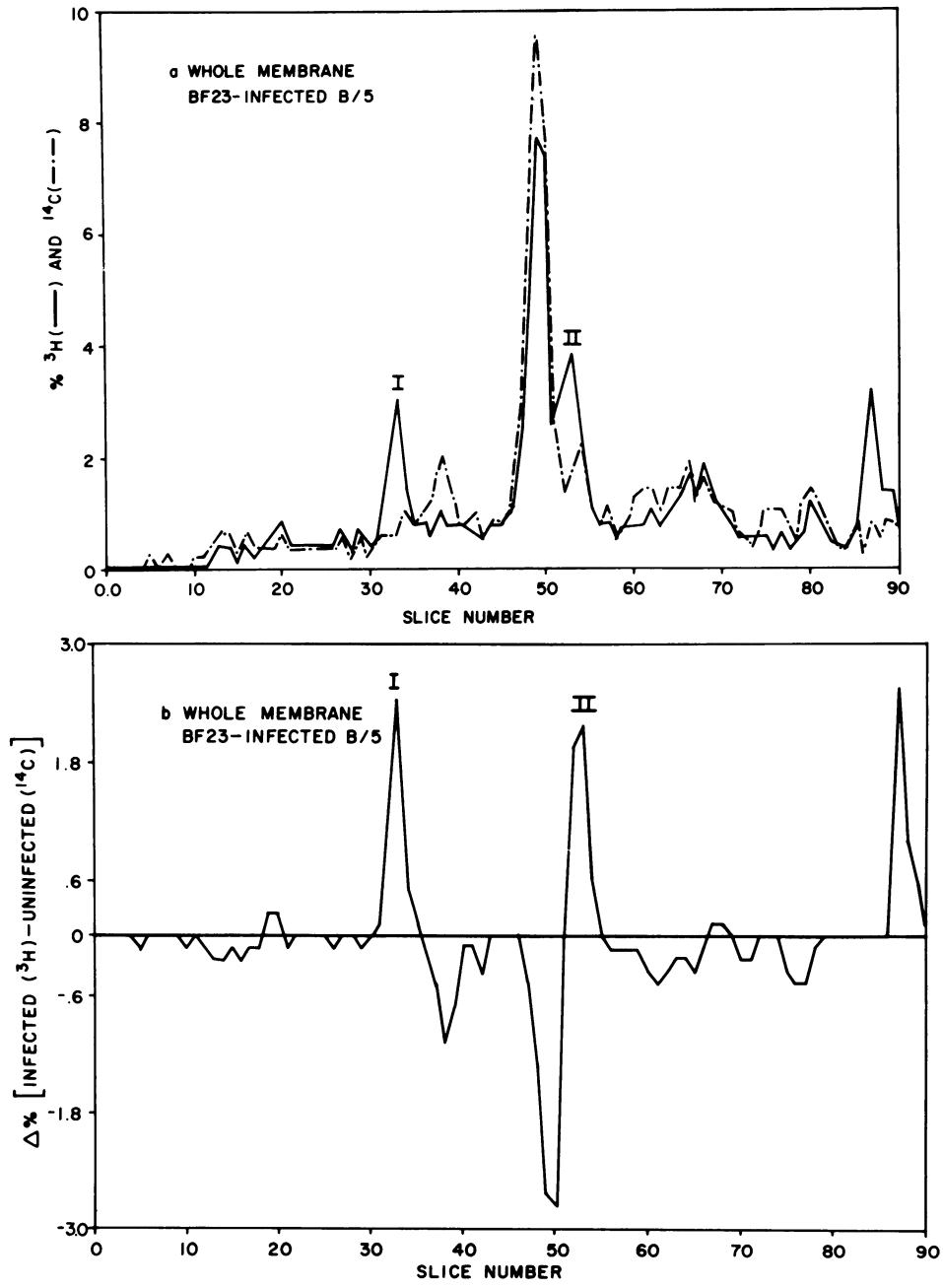

FIG. 1. *Comparison of membrane proteins in uninfected and BF23-infected E. coli B/5. A 100-ml culture of E. coli B/5 in M-9 medium containing 0.5% glucose, 0.05% yeast extract, 5 × 10⁻⁴ M $CaCl_2$, and 10 μg of leucine and tyrosine per ml was grown to a concentration of 8.5 × 10⁸ cells/ml. The cells were infected with 10 BF23 phage/cell and 50 μCi (each) of [³H]leucine and [³H]tyrosine was added. After 12 min the infected cells were mixed with 50 ml of a culture of uninfected E. coli B/5 (8.5 × 10⁸ cells/ml) that had been labeled for the entire growth period in the same medium with 2.5 μCi of [¹⁴C]leucine and 2.5 μCi of [¹⁴C]tyrosine and then treated with 100 μg of chloramphenicol per ml and chilled. The envelopes from this mixture were isolated and divided into two unequal portions. The proteins from the smaller portion (1/3 of the total) were solubilized and subjected to electrophoresis using the Bragg-Hou system, as indicated in the text. The gels were sliced and the counts per minute in each slice was determined. The percentage of total ³H or ¹⁴C in each slice is shown in (a). The dashed line indicates the ¹⁴C-labeled proteins from uninfected cell membranes and the solid line indicates the ³H-labeled proteins from the membranes of T5-infected cells. (b) Difference (³H − ¹⁴C) in the percentage. (c) Control experiment in which the cells were not infected before addition of the ³H for 12 min.*

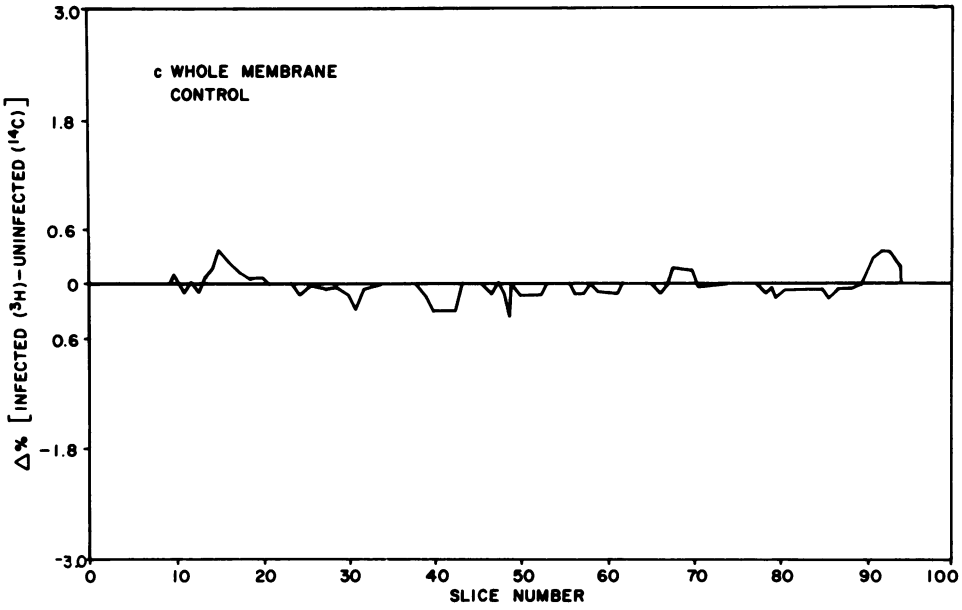

FIG. 1c

fraction, but protein II is not. Other experiments indicate that the amount of protein I in the outer membrane is variable. In T5-infected *E. coli* B in particular, the fraction of the MMP (phage protein I) in the outer membrane is much greater. Protein II is found exclusively in the inner membrane, however (see below).

In the case of BF23-infected *E. coli* JK20, protein II, not visible in the whole membrane fraction, was clearly apparent in the inner membrane. Again, it was completely absent from the outer membrane.

Molecular weight determinations. Figure 3 indicates the apparent molecular weights of proteins I and II. Protein I is found to have a molecular weight of 55,500 and protein II has a molecular weight of 31,000. The molecular weights we calculate for the two major outer envelope proteins are slightly lower than those reported. Rosenbusch (19) calculates a molecular weight of 36,500 for the matrix protein called protein 1 by Schnaitman (24) and protein I by Hindennach and Henning (9). We calculate a weight of slightly less than pepsin, around 35,000. The host membrane protein 3 (Schnaitman's terminology [24]), called protein II by Henning, has been reported to have an apparent molecular weight of 33,000 after heat modification (9). We find it to be ~30,000. These differences may stem from the fact that the mobilities of our markers were obtained from stained gels and the mobilities of the phage and bacterial proteins were obtained from unstained frozen and sliced gels. The lengths of these may be slightly different. These differences may indicate that our phage proteins have molecular weights slightly greater than those indicated here.

Effect of the A1⁻ mutation. BF23 or T5 bacteriophage mutant in the A1 gene cannot inject the complete phage genome or cause breakdown of the host DNA (2). When *E. coli* B/5 is infected for 15 min with a BF23 mutant in the A1 gene, no protein I is produced, confirming results obtained with T5. This data is shown in Fig. 4. Figure 4a is the result of a control experiment using wild-type BF23 and Fig. 4b is the A1⁻ mutant. The lack of "shut-off" of synthesis of host protein, most apparent in the major outer envelope region, is consistent with the A1⁻ phenotype. Synthesis of phage membrane protein II is, in the case of the A1⁻ mutant, masked by the continued synthesis of host protein in this region. Comparison of Fig. 4a and b shows that in the region of slices 48 through 51 the tritium incorporation is not decreased in the mutant, although only a very small difference is apparent in Fig. 4c. Electrophoresis of the Triton-soluble fraction indicates that protein II is present in the A1⁻ mutant (data not shown).

Does protein II occur in T5-infected *E. coli* B? Our previous work on whole membrane fractions of T5-infected *E. coli* has consistently shown only one major area of difference in proteins with molecular weights greater than

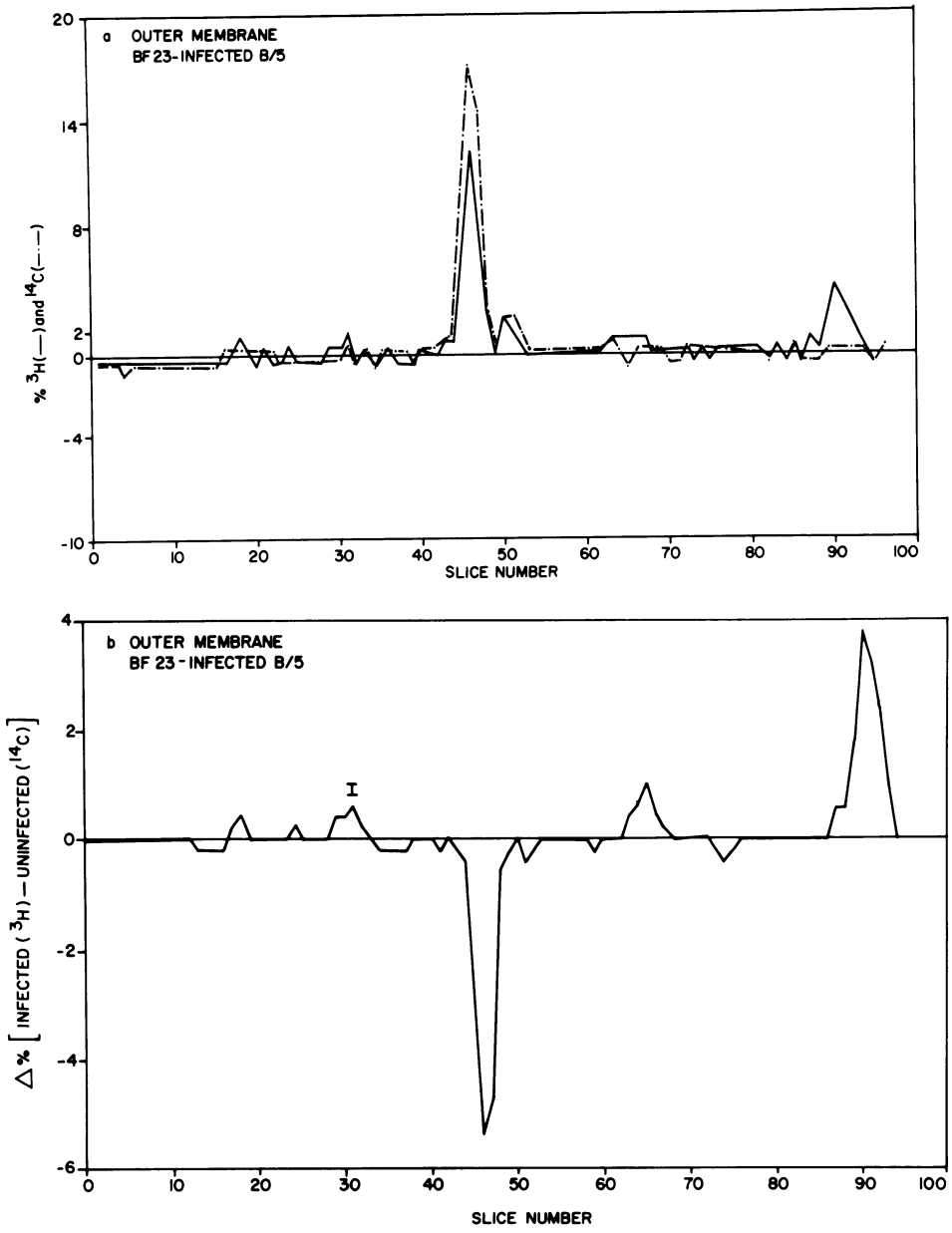

FIG. 2. *Separation of the inner and outer membranes of BF23-infected E. coli B/5. The large (2/3) portion of the membranes isolated in the experiment described in Fig. 1 was extracted with Triton X-100 as described in the text. The Triton-soluble proteins were precipitated with ethanol and prepared for electrophoresis. The Triton-insoluble fraction was washed and prepared for electrophoresis as described. Electrophoresis was with the Bragg-Hou system. (a) Percentage of total ^{3}H (——) and ^{14}C (– · –) in each slice when the Triton-insoluble (outer membrane) fraction was subjected to electrophoresis. (b) Difference in these percentages. (c) Percentage of total ^{3}H (——) and ^{14}C (– · –) in each slice when the Triton-soluble proteins were subjected to electrophoresis, and (d) shows the difference in these percentages.*

10,000 when these are compared with proteins of uninfected cell membranes. Since our work with BF23 indicated the induction of two membrane proteins after infection, we repeated some of our earlier work on T5, this time separating the whole membrane into its inner and outer components. We also used the Bragg-Hou gel system (5) as opposed to the Maizel system

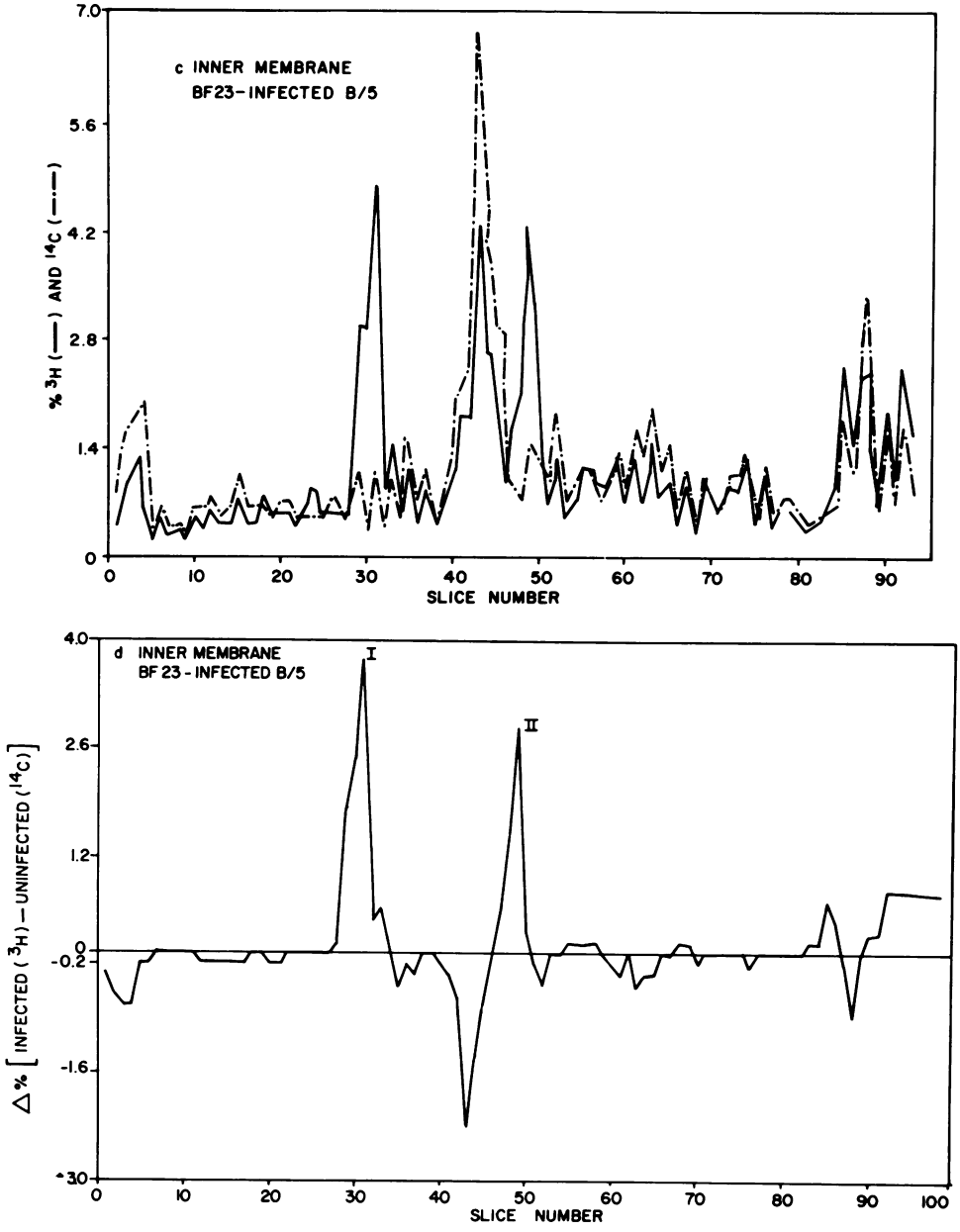

FIG. 2c and d

(16) previously used. In the experiments illustrated, the cells were labeled from 0 to 10 min postinfection. Figure 5a shows the protein profile of the whole membrane fraction of T5-infected *E. coli* B as it appears on Maizel gels. Figure 5c shows the same preparation as it appears after electrophoresis in the Bragg-Hou system. In the Bragg-Hou system there are two peaks of host outer envelope protein (slices 49 through 54 and 56 through 59), whereas in the Maizel system these two peaks are not resolved. Looking at the ^{3}H counts in the Bragg-Hou system, it appears as if the synthesis of these two host proteins is turned off at different rates. In the difference plot (Fig. 5d), there is a small positive difference in this area that does not appear on the Maizel gel plot (Fig. 5b).

Figure 6a shows the differences in the Triton-

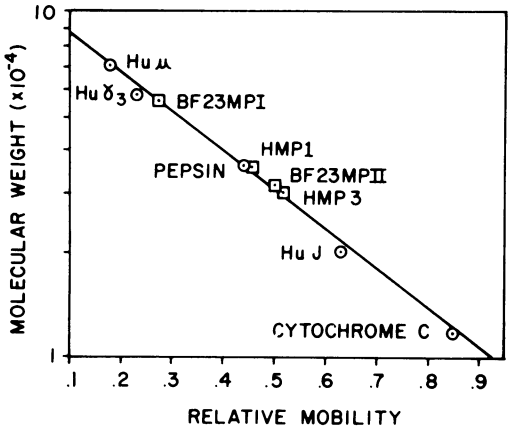

FIG. 3. *Relationship between molecular weight and relative mobility. Samples of human μ immunoglobulin chains (Hu μ[10]), human γ_3 immunoglobulin chains (Hu γ_3 [20]), pepsin (4), methylated human J immunoglobulin chains (Hu J [L. J. McCumber and L. W. Clem, Biochim. Biophys. Acta, in press]), and cytochrome c (26) were prepared for electrophoresis by our standard procedure for preparing membrane proteins. They were subjected to electrophoresis in the Bragg-Hou gel system and stained after 10 h. The relative mobility of the markers was calculated from the distance to the center of the stained bands divided by the distance to the center of the bromophenol blue dye. The relative mobilities of the phage and bacterial membrane proteins were calculated from the distance to the peak number of counts in sliced gels divided by the distance to the center of the bromophenol blue dye. Abbreviations: BF23 MPI, Membrane protein I from BF23-infected B/5; HMPI, host outer membrane protein 1(24); BF23 MPII, membrane protein II from BF23-infected E. coli B/5; HMP3, host outer membrane protein 3(24).*

insoluble fraction of the T5-infected *E. coli* B membranes, and Fig. 6b shows the differences in the Triton-soluble fraction. As was seen in the BF23-infected cells, two peaks of difference are clearly seen in the Triton-soluble fraction, whereas only one major area of difference is seen in the Triton-insoluble fraction. With the T5-infected cells the fraction of phage protein I (T5 MMP) found in the outer membrane appears to be much greater than that found in the outer membrane of BF23-infected cells. The amount of this T5-induced protein in whole membranes is also generally much greater than the analogous BF23 protein.

When the membranes of the T5-infected cells are separated by a physical method, the same results are obtained. This is shown in Fig. 6c and d.

We conclude, therefore, that both T5 and BF23 induce the synthesis of at least two membrane proteins. The differences in the T5 and BF23 system are discussed below.

DISCUSSION

Our previous work with T5-infected *E. coli* showed that a major new protein species appeared associated with the cells' membranes at very early times after infection (6). The protein was found in membranes of cells that had been infected in the presence of chloramphenicol and then sheared in a Waring blender before removal of the chloramphenicol and in *E. coli* that contained the colicinogenic factor, Col Ib, and undergo an abortive infection with T5 (13, 17, 18). This indicated that the membrane protein was probably a pre-early protein. Pre-early proteins of T5 and BF23 are those proteins that are synthesized after the first 8% of the T5 genome enters the cell and are necessary for the entry of the whole T5 genome into the cell (13, 14). They are the only proteins made in the abortively infected Col Ib-containing cells (17). Eight polypeptides, four of which were undetected in the original work (3), are now thought to be synthesized during the pre-early phase of infection (G. Chinnandurai and D. J. McCorquodale, manuscript in preparation). These have been hypothesized to combine in various ways to give the observed pre-early proteins (3, 17). One of the polypeptides having a molecular weight of ~57,000 has been identified as the product of the A1 gene (2, 3).

The molecular weight of the membrane protein we observed in T5-infected cells is between 54,000 and 58,000 (6). As it appeared to be a pre-early protein, it seemed likely that it may have been the A1 gene product. This was confirmed by the use of $A1^-$ and $A2^-$ mutants, both of which can transfer only the initial 8% of the DNA to the cell. The T5 MMP was absent in cells infected with the $A1^-$ mutant but present in cells infected with the $A2^-$ mutant (7). To confirm the fact that the A1 gene controlled the synthesis of a membrane protein, we looked at membrane protein synthesis in BF23-infected *E. coli*.

BF23-infected cells appear to synthesize at least two membrane proteins. These are made very early after infection and in cells that contain the Col Ib factor. Neither is synthesized if the phage is irradiated with UV light prior to infection (data not shown). One of the proteins, BF23 membrane protein I, has a molecular weight of about 55,500 and is not synthesized if an $A1^-$ mutant of BF23 is used. It is, hence, analogous to the MMP of T5-infected cells. It is, however, synthesized in comparatively smaller amounts (percentages) than the T5 MMP. The

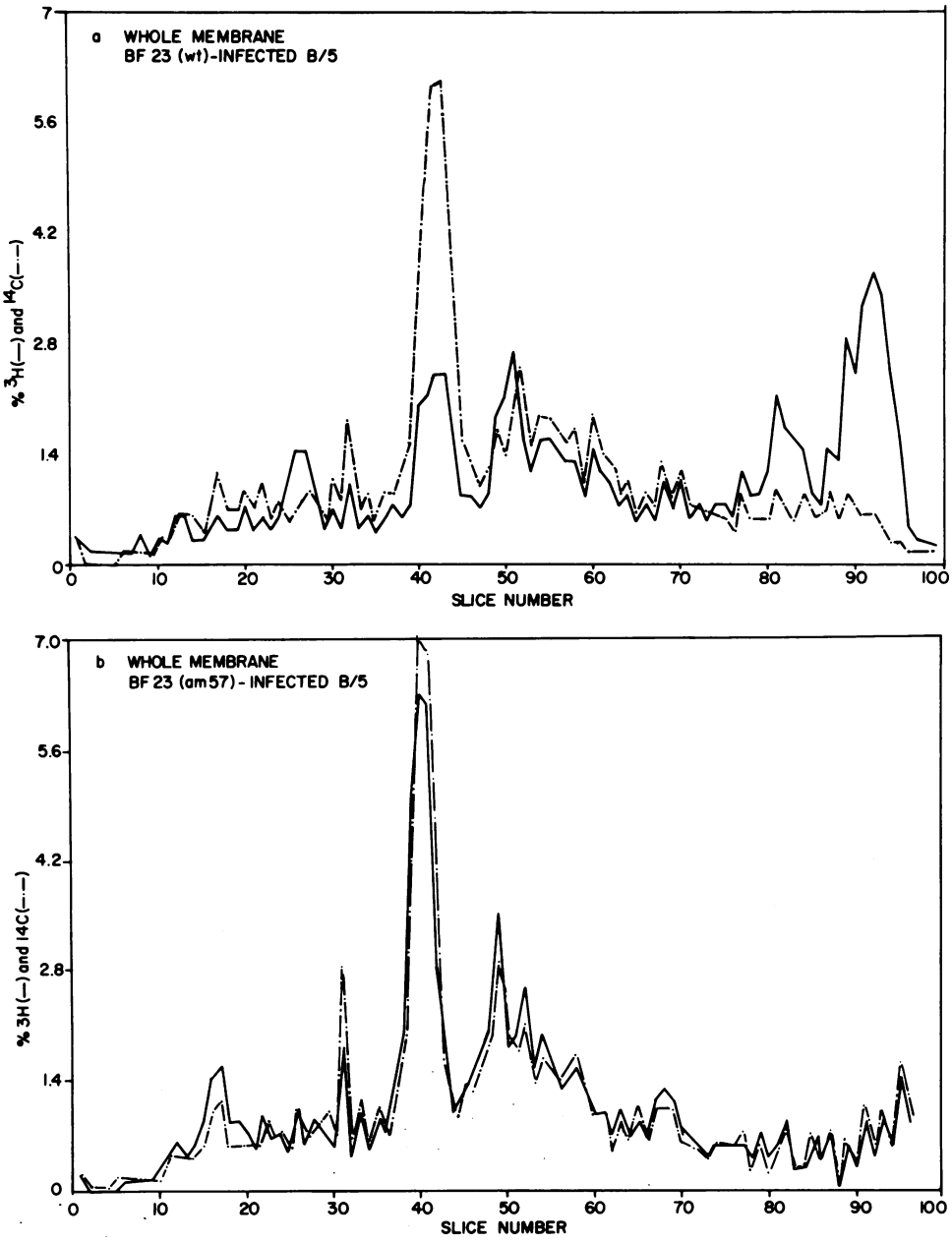

FIG. 4. *Effect of an amber mutation in the A1 gene of BF23. This experiment was performed as in Fig. 1 except that wild-type and A1⁻ mutant phage were used to infect the cells for 15 min. (a) Percentage of ^3H and ^{14}C in each slice when the whole membrane fraction of the wild-type-infected cells was subjected to electrophoresis. (b) Percentage of ^3H and ^{14}C in each slice when the whole membrane fraction of the A1⁻ mutant-infected cells was subjected to electrophoresis. (c) Difference in these percentages. Symbols: ——, wild type; — · —, A1⁻ mutant.*

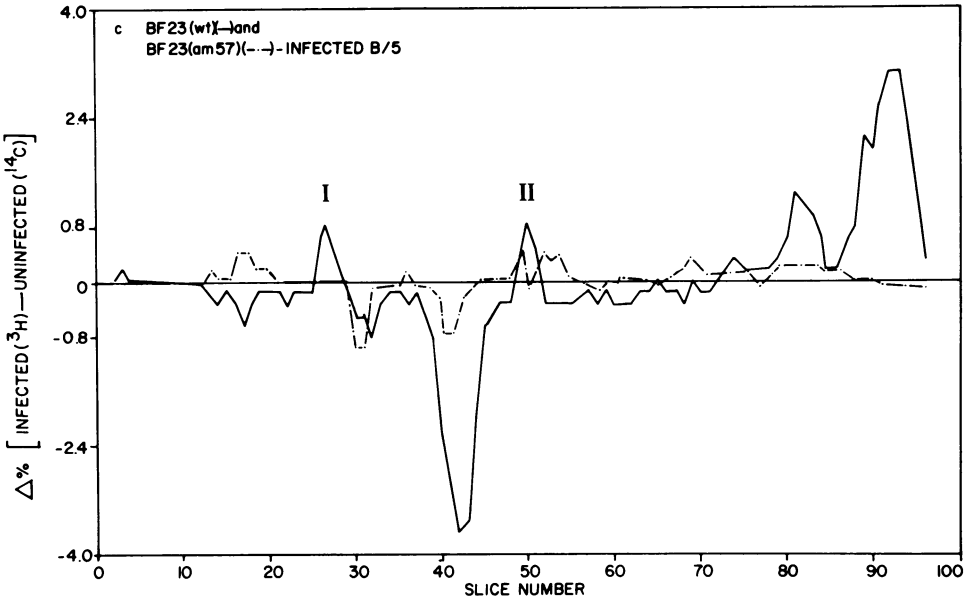

FIG. 4c

BF23 membrane protein I is found primarily associated with the inner membrane of the infected cells, although some does occur in the outer membrane. The T5 MMP, which is present in much greater amounts in the membranes of *E. coli* B, is apparently distributed differently than the analogous BF23 protein, being found in approximately equal amounts in both the inner and the outer membranes. Whether this is due to differences in the phage, the host, or the protein is not known.

In BF23-infected cells a second smaller-molecular-weight species of protein, BF23 membrane protein II, also was observed in the cell membrane. This has a molecular weight of about 30,000 and appears in the membrane in amounts comparable to BF23 membrane protein I. One of the pre-early polypeptides found by McCorquodale has a molecular weight of 29,000 (Chinnandurai and McCorquodale, in preparation) and may correspond to membrane protein II. Our estimated molecular weight may be slightly low (see Results), so this possibility must be investigated further. This smaller protein occurs exclusively in the inner membrane. Further experiments with T5-infected cells showed that an analogous protein also appeared in the inner membrane after T5 infection. It is made in amounts comparable to the amount made in BF23-infected cells, an amount much less than the amount of the A1 product, the MMP, in the T5-infected cell membranes. It was not originally seen, partly because it is sometimes obscured by the host major envelope proteins and partly because it occurred in *E. coli* B in much smaller amounts than the T5 MMP. (See, however, Fig. 3 in reference 6 and Fig. 1b in reference 7.)

It is interesting to note that Schnaitman et al. have reported (25) that a protein of this same approximate molecular weight appears in the Triton-insoluble fraction of *E. coli* strains infected by a lysogenic phage called PA-2. PA-2 has a morphology very similar to that of T5 and BF23 phage.

In *E. coli* K-12 strains infected with T5, the amount of MMP is much smaller than that found in *E. coli* B (6, 7) and is comparable to the amount of BF23 protein I made in *E. coli* B/5. Hence, the T5-*E. coli* B system seems unusual in the amount of MMP or protein I that is produced. We are planning to use this unusual system for studies of membrane protein biosynthesis.

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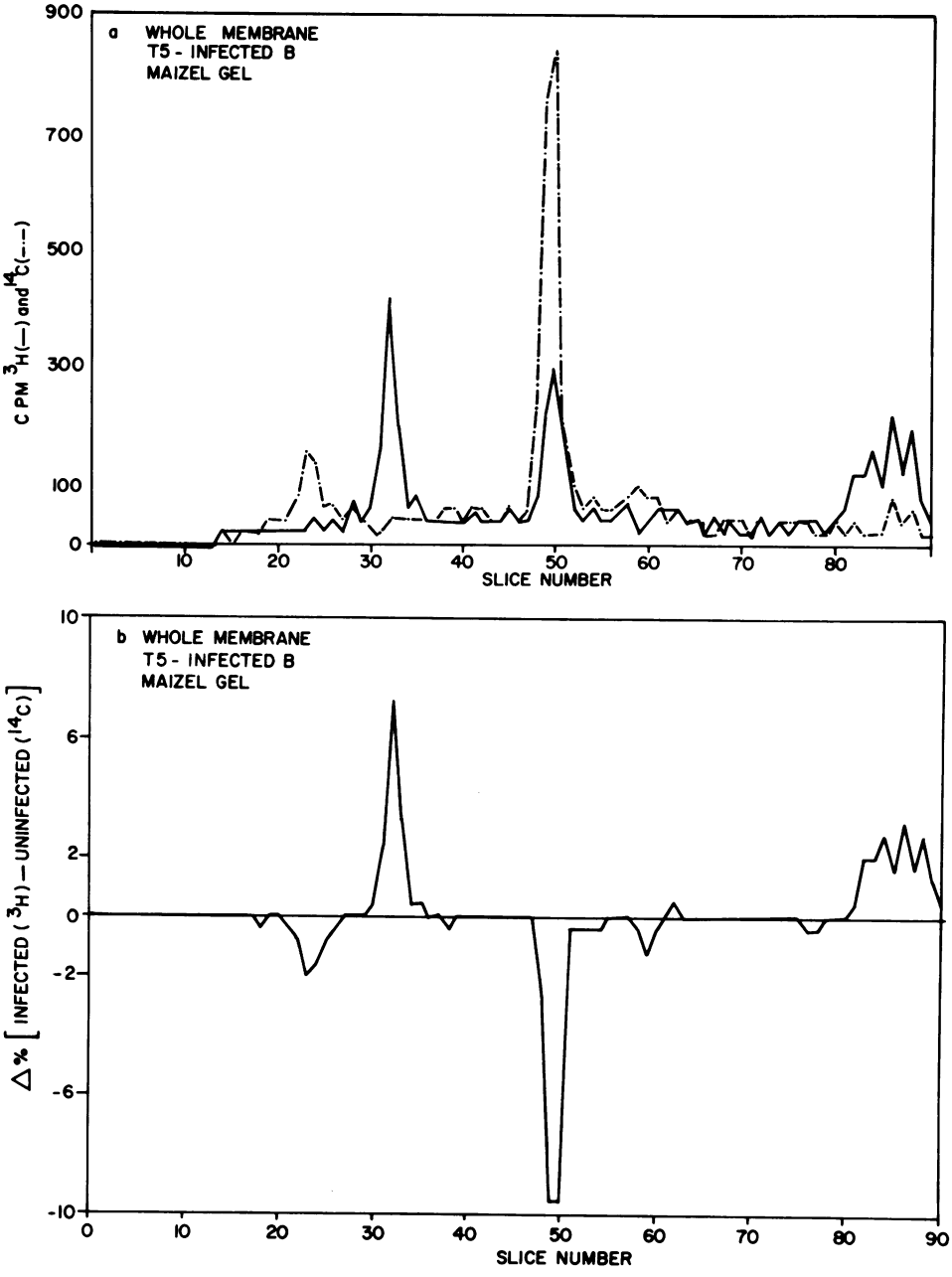

FIG. 5. *Comparison of membrane proteins in uninfected and T5-infected E. coli B. A 100-ml culture of E. coli B in M-9 medium containing 0.5% glucose, 0.05% yeast extract, 5 × 10^{-4} M $CaCl_2$, and 10 μg of leucine and tyrosine per ml was grown to a concentration of 8.5 × 10^{8} cells/ml. The cells were infected with 10 T5 phage/cell, and 50 μCi (each) of [^{3}H]leucine and [^{3}H]tyrosine was added. After 10 min the infected cells were mixed with 50 ml of a culture of uninfected E. coli B (8.5 × 10^{8} cells/ml) in the same medium that had been labeled for the entire growth period with 2.5 μCi of [^{14}C]leucine and 2.5 μCi of [^{14}C]tyrosine and then treated with 100 μg of chloramphenicol per ml and chilled. The envelopes from this mixture were isolated, and the proteins were solubilized and subjected to electrophoresis in either the Maizel (a and b) or Bragg-Hou (c and d) system. The gels were sliced, and the counts per minute in each slice were determined. The dashed line (a and c) indicates the ^{14}C-labeled proteins from uninfected cell membranes, and the solid line indicates the ^{3}H-labeled proteins from the membranes of T5-infected cells.*

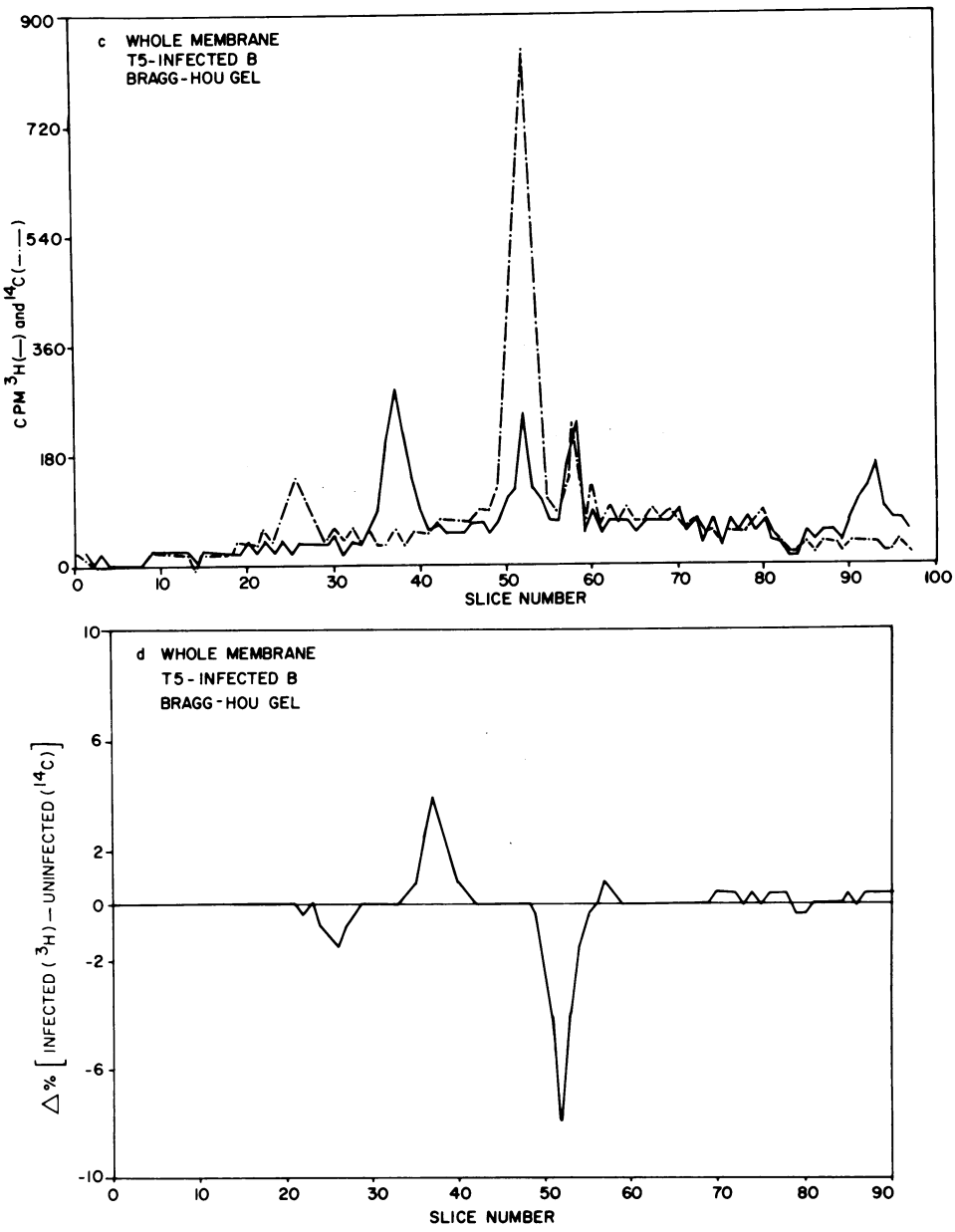
c WHOLE MEMBRANE
T5-INFECTED B
BRAGG-HOU GEL
CPM ^{3}H(—) and ^{14}C(- - -)
SLICE NUMBER
d WHOLE MEMBRANE
T5-INFECTED B
BRAGG-HOU GEL
Δ% [INFECTED (^{3}H) — UNINFECTED (^{14}C)]
SLICE NUMBER

FIG. 5c and d

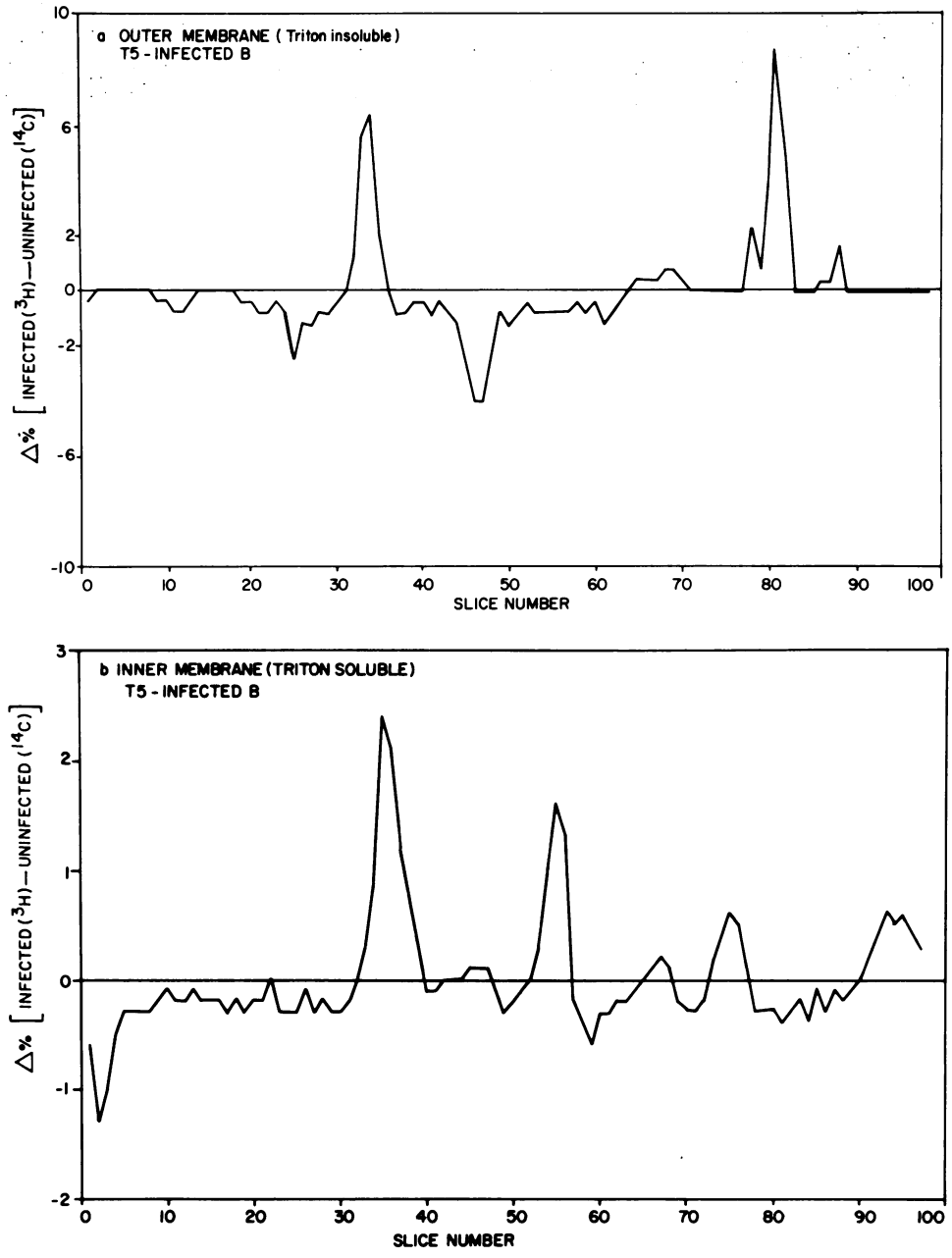

FIG. 6. *Comparison of membrane proteins in the inner and outer membranes of uninfected and T5-infected E. coli B. A 200-ml culture of E. coli B in M-9 medium containing 0.5% glucose, 0.05% yeast extract, 5 × 10^{-4} M $CaCl_2$, and 10 μg of leucine and tyrosine per ml was grown to a concentration of 8.5 × 10^8 cells/ml. The cells were infected with 10 T5 phage/cell, and 50 μCi (each) of [^{3}H]leucine and [^{3}H]tyrosine was added. After 10 min the infected cells were mixed with 100 ml of a culture of uninfected E. coli B (8.5 × 10^8 cells/ml) in the same medium that had been labeled for the entire growth period with 2.5 μCi of [^{14}C]leucine and 2.5 μCi of [^{14}C]tyrosine and then treated with 100 μg of chloramphenicol per ml and chilled. The cells were collected, washed, and resuspended in distilled water. They were incubated at 37°C for 30 min in distilled water. The cells were then collected again and resuspended in Tris buffer (pH 7.8) containing 1 mM EDTA. The envelopes from these cells were isolated and divided into three portions. From one of these the proteins were solubilized and subjected to electrophoresis in the Bragg-Hou or Maizel gel system. The remaining two portions were separated into outer and inner membranes either by the Triton extraction method or by sucrose density gradient centrifugation, as described in the text, and their proteins were solubilized and subjected to electrophoresis as above. Difference plots of membrane protein profiles of Triton-insoluble and -soluble fractions are shown in (a) and (b). A difference plot of proteins taken from a portion of the gradient with a density of 1.22 is shown in (c) and those from a fraction with a density of 1.15 are shown in (d). These represent outer and inner membrane, respectively.*

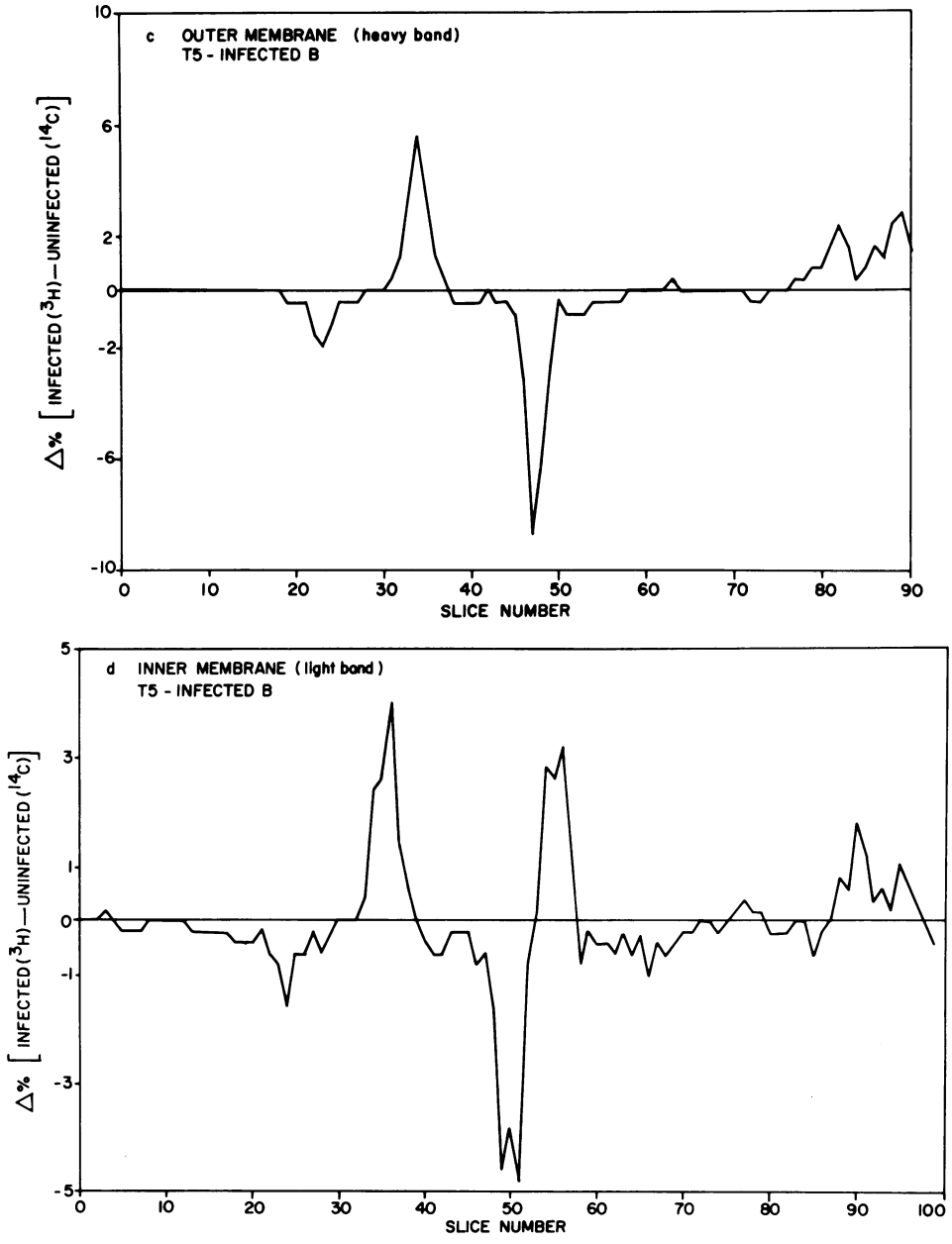
c OUTER MEMBRANE (heavy band)
T5 - INFECTED B
Δ% [INFECTED (3H) — UNINFECTED (14C)]
10
6
2
0
-2
-6
-10
0
10
20
30
40
50
60
70
80
90
SLICE NUMBER
d INNER MEMBRANE (light band)
T5 - INFECTED B
Δ% [INFECTED (3H) — UNINFECTED (14C)]
5
3
1
0
-1
-3
-5
0
10
20
30
40
50
60
70
80
90
100
SLICE NUMBER

FIG. 6c and d

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