Proc. Natl. Acad. Sci. USA
Vol. 74, No. 4, pp. 1388–1392, April 1977
Biochemistry

Biochemical characterization of a mutant lipoprotein of *Escherichia coli*

(membrane/murein/precursor/lipoprotein gene)

HENRY C. WU, CATHERINE HOU, JIM J. C. LIN, AND DANIEL W. YEM

Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06032

Communicated by Herman M. Kalckar, December 29, 1976

ABSTRACT **A lipoprotein mutant of *E. coli* K-12 has been characterized. The mutant lipoprotein was found to differ from the wild-type lipoprotein in the following respects: (*i*) it is present in an appreciable amount in the soluble fraction (275,000 × *g* supernatant); (*ii*) it lacks the covalently-linked diglyceride; (*iii*) it contains an unmodified cysteine which can be carboxymethylated *in vitro;* (*iv*) it undergoes dimerization and the dimer can be converted into monomeric form by reduction with 2-mercaptoethanol; (*v*) both the monomeric form and especially the dimeric form of the mutant lipoprotein migrate more slowly than the corresponding forms of wild-type lipoprotein in sodium dodecyl sulfate/urea polyacrylamide gel electrophoresis; and (*vi*) the mutant lipoprotein is not assembled into the murein sacculi, and this results in a greatly reduced amount of bound-form lipoprotein in the mutant. These data strongly suggest that the mutation has affected the primary structure of lipoprotein, in such a way that it is not modified normally, leading to the production of a structurally-altered lipoprotein deficient in covalently-linked lipid as well as a defective assembly of the altered lipoprotein into the rigid layer of the cell envelope.**

The cell envelopes of *Escherichia coli* contain a major protein, the murein lipoprotein as discovered by Braun and his coworkers (1). This lipoprotein is present in the outer membrane of the cell envelope in two forms, free and bound; the bound form is attached to the *meso*-diaminopimelic acid residues in the murein sacculus through the ϵ-NH_2 group of the COOH-terminal lysine of the lipoprotein (2–4). The primary structure of this lipoprotein has been determined (5). The NH_2-terminus of the lipoprotein is a novel lipoamino acid with three fatty-acyl residues covalently attached to glycerylcysteine. Two of the fatty acids are linked to the hydroxyl groups of the glycerylcysteine by ester linkages, and the third is attached through amide linkage to the α-NH_2 group of glycerylcysteine (6).

In an attempt to define the biochemical mechanisms for the biosynthesis and assembly of murein lipoprotein in *E. coli* (7), we have isolated *E. coli* mutants defective in the biosynthesis and/or assembly of murein lipoprotein. In a previous report, we presented preliminary evidence for the presence of a structurally-altered lipoprotein in one of the mutants we have isolated (8). This mutant is strikingly different from the wild-type strain by its extremely low content of the murein-bound lipoprotein (bound form). In this paper, we present further evidence that suggests this mutant is affected in the structural gene for the murein lipoprotein.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The *E. coli* strains used in the present study were wild-type strain E600 (F^- ara_{am} $lac125_{am}$ *gal*K $U4_{am}$ *gal*E trp_{am} *Su*IIIA81_{ts} *his pro* *mlp*A^+) and mutant 3 (F^- ara_{am} $lac125_{am}$ *gal*K $U4_{am}$ *gal*E trp_{am} *Su*IIIA81_{ts} *his pro* *mlp*A^-) as described (8). *E. coli* strains JE5512 (Hfr Cavalli *man*$^-$ *lpm*$^+$ *pps*$^-$) and JE5511 (Hfr Cavalli *man*$^-$ *lpm*$^-$ *pps*$^-$) were the generous gift of Y. Hirota, National Institute of Genetics, Japan. Media used in the present study included protease peptone beef extract broth and M9 minimal medium (7).

Biochemical Techniques. Procedures used for the labeling of bacterial cells, preparation of cell envelope, and the identification of both the free and bound forms of murein lipoprotein by both immunological and biochemical techniques have been described (7, 8). Sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis was carried out as described (9). Polyacrylamide gel electrophoresis was also carried out in $NaDodSO_4$ and urea according to the procedure of Swank and Munkres (10).

Performic acid oxidation of the purified free form of lipoprotein was carried out according to Hirs (11). Hydrolysis of lipoprotein was carried out in 6 M HCl at 105° for 20 hr in sealed, evacuated tubes. Amino acid analysis was performed with a Beckman model 120 C amino acid analyzer equipped for determination of amino acids in the range of 7.5–30 nmol (12). An acidic amino acid, migrating between cysteic acid and L-aspartic acid, was assumed to be glycerylcysteine sulfone.

Carboxymethylation of Envelope Proteins. Envelope proteins labeled with [^{3}H]arginine were solubilized with 0.4 ml 0.01 M sodium phosphate buffer (pH 7.0) containing 1% $NaDodSO_4$ and 1% 2-mercaptoethanol. After incubation at 37° for 2 hr, the envelope proteins were precipitated with acetone at a final concentration of 80% (vol/vol). The precipitate was recovered by centrifugation at 12,000 × *g* for 10 min and washed once with 2 ml of 80% acetone. The washed precipitate was solubilized in 0.4 ml of 0.01 M sodium phosphate (pH 7.0) containing 1% $NaDodSO_4$. [^{14}C]Iodoacetic acid (16 μCi, specific activity of 6 μCi/μmol) was added to the reduced envelope proteins. The reaction mixture was incubated at 37° for 2 hr in a test tube wrapped with aluminum foil. The reaction was terminated by the addition of 20 μl of 2-mercaptoethanol. Acetone (1.6 ml) was added to precipitate the envelope proteins. The acetone precipitate was washed once with 80% acetone and then solubilized in 0.01 M sodium phosphate (pH 7.0) containing 1% $NaDodSO_4$.

Chemicals and Radiochemicals. All chemicals were of reagent grade and were purchased from commercial sources. Radioactive chemicals used in the present study included [2-^{3}H(*N*)]glycerol (200 mCi/mmol), L-[3-^{3}H(*N*)]arginine (27 Ci/mmol), L-[*U*-^{14}C]arginine (309 mCi/mmol) and iodo[*1*-^{14}C] acetic acid (17 mCi/mmol) were purchased from either New England Nuclear Corp. or Schwarz/Mann.

RESULTS

Preliminary Evidence for the Presence of Structurally Altered Lipoprotein in Mutant 3. Two preliminary observations suggested that the lipoprotein in mutant 3 is structurally different from that in the parental strain. While the isolation

Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

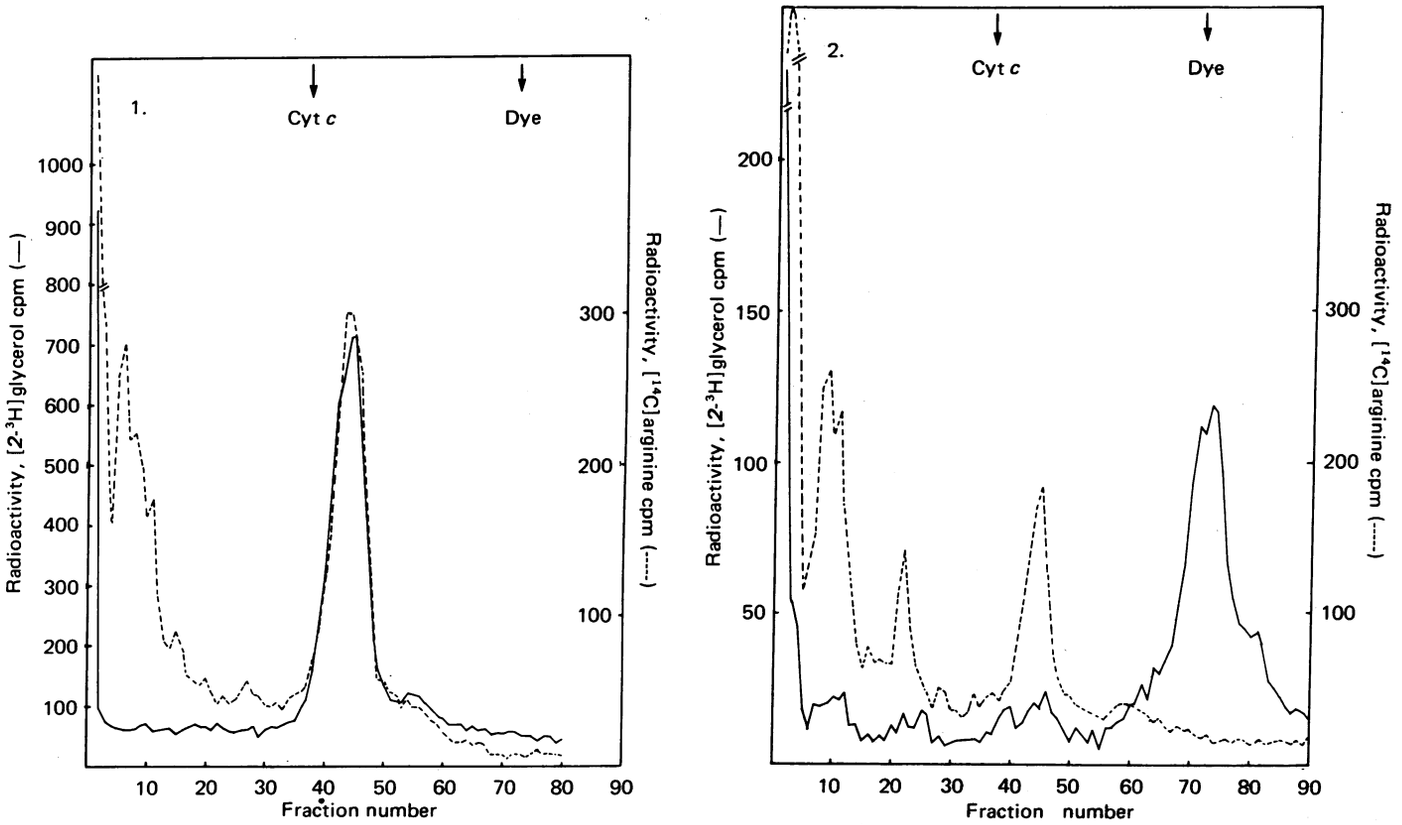

FIG. 1. $NaDodSO_4$/urea gel electrophoresis of immunoprecipitates prepared from cell envelopes of wild-type strain E600 labeled with both [2-^{3}H]glycerol and [^{14}C]arginine. Cell envelope was delipidated with $CHCl_3/CH_3OH$ (2/1, vol/vol) mixture, solubilized with 1% $NaDodSO_4$ in 10 mM Na phosphate (pH 7.0), and then subjected to immunoprecipitation with antiserum against lipoprotein. The immunoprecipitate was analyzed by $NaDodSO_4$/urea gel electrophoresis (12.5% acrylamide, 1/10 crosslinkage) (10). Arrows show the position of the molecular weight marker, cytochrome *c* (cyt *c*), and the dye.

FIG. 2. $NaDodSO_4$/urea gel electrophoresis of immunoprecipitate prepared from cell envelope of mutant 3 labeled with both [2-^{3}H]glycerol and [^{14}C]arginine. Other details are given in the legend to Fig. 1.

of murein sacculi provides a simple way to obtain pure lipoprotein in its bound form, the extremely low content of the bound-form lipoprotein in mutant 3 makes this approach infeasible. We therefore attempted to purify the free-form lipoprotein from mutant 3 according to Inouye *et al.* (13). In contrast to the wild-type lipoprotein which was soluble in a *n*-butanol/$NaDodSO_4$ buffer mixture [Tris·acetate, 0.02 M/$NaDodSO_4$, 1.7%/EDTA, 8.5/mM *n*-BuOH, 12.8% (vol/vol)] at pH 5.2 incubated in an ice bath, the mutant lipoprotein was precipitated under the same conditions, as revealed by the $NaDodSO_4$/gel electrophoresis (data not shown). This apparent difference in the solubility of mutant lipoprotein compared with the wild type was confirmed by Ouchterlony test of both the *n*-BuOH/$NaDodSO_4$-soluble fraction and the precipitate fraction, and by immunodiffusion after $NaDodSO_4$/gel electrophoresis (data not shown). Second, a significant fraction of mutant lipoprotein (20% of total lipoprotein antigen as measured by Ouchterlony test of serially diluted fractions) was found in the 275,000 × *g* cell supernatant fraction, in contrast to the quantitative recovery of wild-type lipoprotein (100% of lipoprotein antigen), in the 275,000 × *g* pellet. These apparent differences in the solubility properties of lipoproteins from the mutant and wild-type strains may reflect a structural alteration of the lipoprotein in this mutant.

There is no evidence that the membrane-bound lipoprotein in mutant 3 differs from that found in the soluble fraction. The lipoproteins in both wild-type strain E600 and mutant 3 were found exclusively in the outer membrane of the cell envelope (D. W. Yem and H. C. Wu, unpublished data). This is of some interest because the membrane-bound lipoprotein in mutant 3 can be partially solubilized either with 1% Sarkosyl in 10 mM Na phosphate (pH 7.0) or with 6 M guanidine·HCl in 0.1 M Tris·HCl (pH 8.0) containing 0.01 M EDTA, in contrast to that of the wild-type lipoprotein. Both of these two treatments have previously been shown to preferentially solubilize cytoplasmic membrane proteins from the cell envelope (14, 15).

While we observed striking difference in the amount of murein-bound lipoprotein between the wild-type and the mutant strain, there was only a small and variable increase (50–100%) in the amount of free form of lipoprotein in the mutant, as compared with that of the wild-type strain. This was shown both by double-isotope labeling experiment and by semiquantitative assay of lipoprotein antigen by using Ouchterlony immunodiffusion test.

Mutant Lipoprotein Is Deficient in Covalently Linked Diglyceride. It has been shown previously that the covalently-linked diglyceride at the NH_2-terminal cysteine of the lipoprotein can be specifically labeled with [2-^{3}H]glycerol (6, 7). Both the wild type and mutant 3 were labeled with both [2-^{3}H]glycerol and [^{14}C]arginine in M9 lactate medium supplemented with the required amino acids. Phospholipids were extracted from the cell envelope fractions and the specific activities of phospholipid labeled with [2-^{3}H]glycerol were found to be 3.14×10^5 cpm/μmol of phospholipid phosphate for wild-type strain E600 and 3.75×10^5 cpm/μmol phospholipid phosphate for mutant 3, respectively. The specific activities of [^{14}C]arginine cell envelope proteins were determined to be 9.2×10^5 cpm/mg of protein for wild-type strain E600 and 1.17

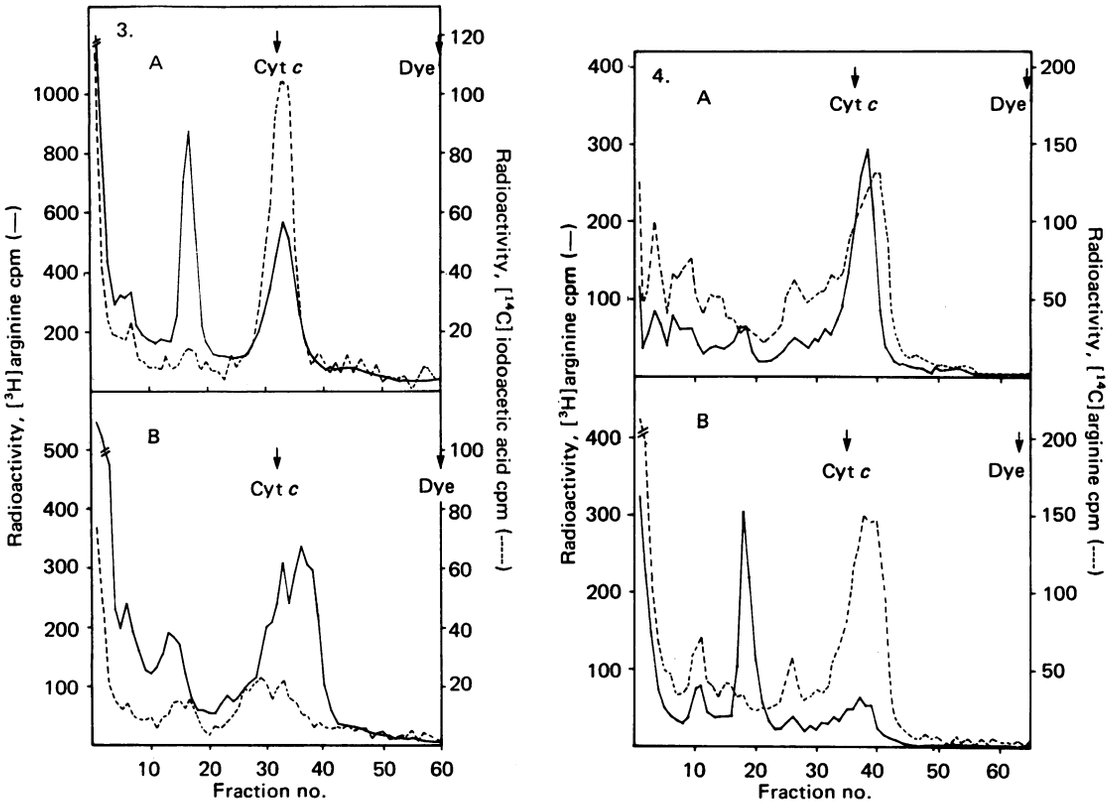

FIG. 3. Carboxymethylation of the mutant lipoprotein. The details for carboxymethylation of envelope proteins labeled with [^{3}H]arginine are given in *Materials and Methods*. [^{14}C]Carboxymethylated envelope proteins were solubilized in 0.01 M sodium phosphate (pH 7.0) containing 1% $NaDodSO_4$ and subjected to immunoprecipitation. The immunoprecipitates were analyzed by $NaDodSO_4$/urea gel electrophoresis. (*A*) Immunoprecipitate from mutant 3; (*B*) immunoprecipitate from wild-type strain E600..

FIG. 4. Change in the electrophoretic mobility of mutant lipoprotein by reduction with 2-mercaptoethanol. Immunoprecipitates were obtained by treatment of $NaDodSO_4$-solubilized cell envelopes from mutant 3 labeled with [^{3}H]-arginine and wild-type strain E600 labeled with [^{14}C]arginine with antiserum against lipoprotein. The immunoprecipitates were analyzed by $NaDodSO_4$/urea gel electrophoresis with or without prior reduction with 2-mercaptoethanol. (*A*) Immunoprecipitates treated with $NaDodSO_4$/urea buffer containing 2-mercaptoethanol prior to electrophoresis; (*B*) immunoprecipitates treated with $NaDodSO_4$/urea buffer containing *no* 2-mercaptoethanol prior to electrophoresis.

$\times 10^6$ cpm/mg of protein for mutant 3, respectively. $NaDodSO_4$-solubilized envelope proteins were subjected to immunoprecipitation with antiserum against lipoprotein and the immunoprecipitates were analyzed by $NaDodSO_4$/urea gel electrophoresis which provided better separation of lipoprotein from phospholipids. Figs. 1 and 2 show that lipoprotein in strain E600 was readily labeled with both [2-^{3}H]glycerol and [^{14}C]arginine, whereas the lipoprotein in mutant 3 was not labeled with [2-^{3}H]-glycerol. The ^{3}H-labeled peak migrating with the tracking dye in Fig. 2 was most likely [2-^{3}H]glycerol labeled phospholipids bound to the lipoprotein noncovalently.

Mutant Lipoprotein Contains an Unmodified Cysteine Residue. The data shown in Fig. 2 strongly suggested that the mutant lipoprotein should contain an unmodified cysteine residue. This prediction was verified by the experiments described below.

(*i*) Mutant lipoprotein could be carboxymethylated *in vitro* with [^{14}C]iodoacetic acid under conditions optimal for the reaction of iodoacetic acid with free SH groups (16); under the same condition, the wild-type lipoprotein was not labeled (Fig. 3). (*ii*) Mutant lipoprotein was preferentially adsorbed by an activated thiol-Sepharose 4B column and was eluted with buffer containing 20 mM L-cysteine (data not shown). (*iii*) Mutant lipoprotein undergoes dimerization in the absence of an SH-reducing agent. Suzuki *et al.* have recently isolated and partially characterized an *E. coli* mutant from which the lipoprotein was recovered as a dimer with an apparent molecular weight of 15,000. This dimer form of lipoprotein can be converted to the monomer form with a molecular weight of about 7500 by reduction with 2-mercaptoethanol (17). Fig. 4 shows that lipoprotein from mutant 3 can also undergo dimerization in the absence of SH-reducing agent. While both the reduced and unreduced lipoprotein from the wild-type strain showed the same electrophoretic mobilities in $NaDodSO_4$/urea gel, the reduced form of mutant lipoprotein showed a greatly increased mobility in $NaDodSO_4$/urea gel compared with the unreduced form. This is consistent with an intermolecular disulfide bridge between the unmodified cysteine present in the mutant lipoprotein.

(*iv*) Direct amino acid analysis of lipoprotein purified from wild-type strain E600 and mutant 3 showed a marked reduction in the content of glycerylcysteine in the mutant lipoprotein as compared to that of the wild type (Table 1).

Mutant Lipoprotein May Be of Higher Molecular Weight Than That of the Wild Type. We have previously shown a slower migration of the mutant lipoprotein compared with that of the wild type, by polyacrylamide gel electrophoresis in the presence of both $NaDodSO_4$ and urea (8). This small but significant difference in the electrophoretic mobility between mutant lipoprotein and the wild-type form was attributed to one of two possibilities which are not mutually exclusive: (*i*) mutant lipoprotein may bind less $NaDodSO_4$ than that of the wild-type strain due to its deficiency in the covalently-linked diglyceride; or (*ii*) mutant lipoprotein may actually be larger in size than the wild-type lipoprotein with extra amino acids at either the NH_2 terminus, the COOH terminus or both.

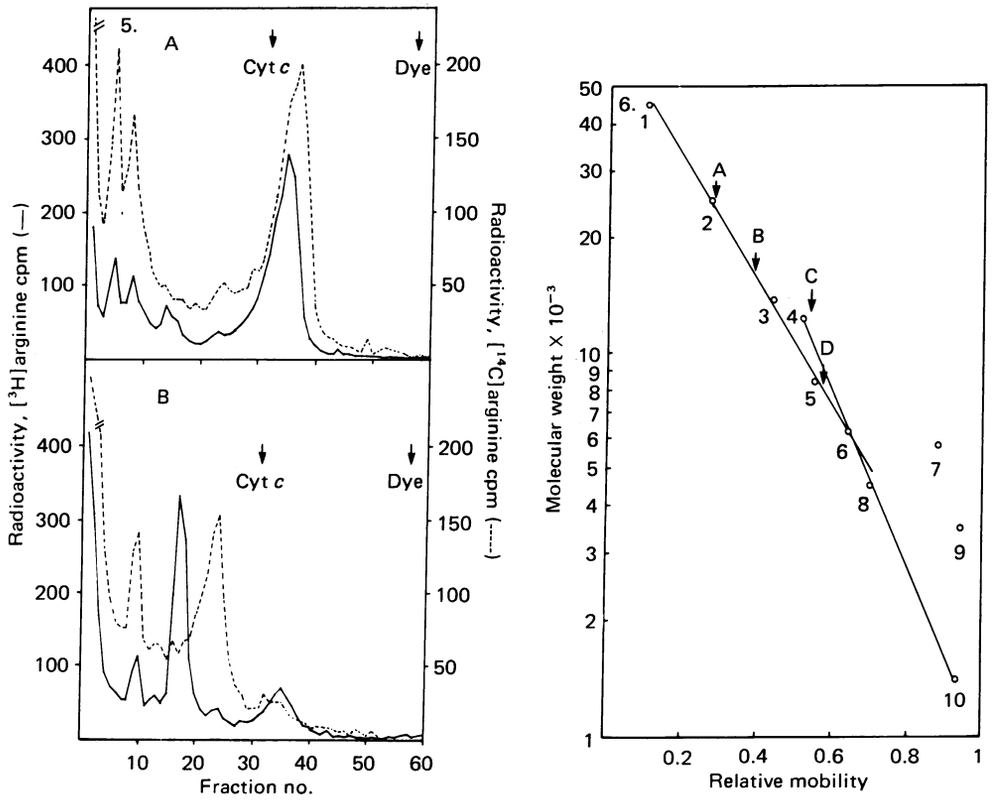

FIG. 5. $NaDodSO_4$/urea gel electrophoresis of mutant 3 lipoprotein (labeled with [^{3}H]arginine) compared with *lpm*$^-$ lipoprotein of strain JE5511 labeled with [^{14}C]arginine. Immunoprecipitates were prepared from the mixed cell envelope and analyzed by $NaDodSO_4$/urea gel electrophoresis. (*A*) Immunoprecipitates treated with $NaDodSO_4$/urea buffer containing 2-mercaptoethanol prior to electrophoresis; (*B*) immunoprecipitates treated with $NaDodSO_4$/urea buffer containing *no* 2-mercaptoethanol prior to electrophoresis.

FIG. 6. Determination of molecular weight of lipoprotein in mutant 3 by $NaDodSO_4$/urea gel electrophoresis. A plot of the logarithm of molecular weights for the marker proteins versus the relative mobility to the tracking dye (pyronin Y) in the gel provided the standard curve. Molecular weight standards used included: (*1*) ovalbumin; (*2*) chymotrypsinogen; (*3*) ribonuclease A; (*4*) cytochrome *c*; (*5*) histone; (*6*) bovine trypsin inhibitor; (*7*) insulin; (*8*) corticotropin; (*9*) glucagon, and (*10*) bacitracin. The arrows representing the mobility of lipoproteins from various strains were: (*A*) dimer of mutant 3 lipoprotein; (*B*) dimer of *lpm*$^-$ mutant lipoprotein; (*C*) monomer of mutant 3 lipoprotein; and (*D*) monomer of *lpm*$^-$ lipoprotein as well as wild-type lipoprotein including strain E600, respectively.

To investigate the second possibility, we compared the lipoprotein from mutant 3 with *lpm*$^-$ mutant of Suzuki *et al.*, because the *lpm*$^-$ strain was reported to contain lipid-deficient lipoprotein (17). Fig. 5 shows both the dimeric and the monomeric forms of lipoprotein from mutant 3 migrated more slowly than the corresponding dimer and monomer lipoprotein in the *lpm*$^-$ mutant. Based on the molecular weight calibration curve shown in Fig. 6, the apparent molecular weight of the lipoprotein in mutant 3 would appear to be approximately 9,000–11,000.

Table 1. Content of glycerylcysteine and other sulfur-containing amino acids in the lipoproteins from wild type and mutant 3*

Strain	Experiment	Mol/mol lipoprotein: Glyceryl-cysteine sulfone†	Cysteic acid	Methionine sulfone
E600	1	0.66	0.23	1.58
	2	0.57	0.22	1.74
Mutant 3	1	0	0.87	1.93
	2	0	1.08	1.94

* Purification and characterization of mutant lipoprotein will be described elsewhere (D. R. Howlett, J. C. Lin, and H. C. Wu, manuscript in preparation).

† Determination of the content of glycerylcysteine in lipoprotein was carried out by amino acid analysis of performic acid oxidized lipoprotein as described in *Materials and Methods*. The calculations were based on the assumption that the color yield of the glycerylcysteine sulfone in the ninhydrin reaction was the same as that of methionine sulfone.

While the lipoprotein in the *lpm*$^-$ mutant *might be lipid-deficient*, its assembly into the murein sacculi appeared to be unaffected (17). The data in Table 2 show a striking difference in the assembly of murein-bound lipoprotein in mutant 3 compared with that of the *lpm*$^-$ mutant. It strongly suggests that there is structural difference(s) between the lipoprotein from mutant 3 and that from *lpm*$^-$ mutant, which is responsible for the defective assembly of murein-bound lipoprotein in mutant 3.

Final proof of our working hypothesis that the mutant lipoprotein contains extra amino acids at either the NH_2-terminus, the COOH terminus or both, awaits elucidation of the primary structure of the mutant lipoprotein. Automated Edman degradation studies clearly indicate that the N terminus of the mutant lipoprotein is blocked (J. Ozols, C. Gerard, H. C. Wu, and D. R. Howlett, unpublished data). Carboxypeptidase B treatment releases lysine and arginine from the wild-type lipoprotein but only lysine from the mutant lipoprotein (J. Ozols, H. C. Wu, and D. R. Howlett, unpublished data). In addition, amino acid analysis has revealed significant and reproducible differences between the mutant lipoprotein and that of the wild type. The mutant lipoprotein contains extra isoleucine, leucine,

Table 2. Relative rate of appearance of murein-bound lipoprotein in mutant 3 as compared with wild-type strain E600 and in strain JE5511 (*lpm*$^-$), respectively*

Fraction	$^3H/^{14}C$ ratio	
	[^{3}H]Arg mutant 3/ [^{14}C]Arg E600	[^{3}H]Arg mutant 3/ [^{14}C]Arg JE5511
Crude extract†	1.00	1.00
Soluble fraction	1.05	1.02
Cell envelope	1.05	0.92
$NaDodSO_4$-soluble envelope proteins	1.10	0.93
Immunoprecipitation supernatant	1.07	0.93
Immunoprecipitate	1.07	0.93
Murein-bound lipoprotein	0.01	0.04

* Relative rate of appearance of murein lipoprotein was determined by a double-isotope labeling experiment according to the protocol described in detail (8).

† The $^3H/^{14}C$ ratio of the crude extract was arbitrarily set at 1.00.

glutamic acid, lysine, and possibly glycine compared with that found in the wild type. Due to incomplete removal of detergent ($NaDodSO_4$ or Triton X-100) from the purified lipoprotein, analysis of the fatty-acid content in the mutant lipoprotein has been unsuccessful.

Genetic Characterization of Lipoprotein Mutation in Mutant 3. By P1-mediated transduction, we have recently determined the map position of the mutation in this lipoprotein mutant on the *E. coli* chromosome (D. W. Yem and H. C. Wu, manuscript in preparation). Like the *lpm* mutant (17), this mutation is cotransducible with *aroD*. In addition, we have found that all three of the biochemical characteristics of the mutant lipoprotein (dimerization in the absence of mercaptoethanol, lipid deficiency, and greatly reduced content of murein lipoprotein) are simultaneously transduced to the recipient.

DISCUSSIONS

Recent studies on the biosynthesis and assembly of murein lipoprotein suggest a series of biochemical reactions which constitute novel and extensive posttranslational modification of the translation product (18). It now appears very likely that the primary translation product of the lipoprotein gene is a polypeptide of an apparent molecular weight of 15,000. One of two reactions is likely to follow: (*i*) transfer of the diglyceride moiety from glycerophosphatides to the sulfhydryl group of the cysteine residue which ultimately becomes the NH_2-terminal amino acid of the lipoprotein; and (*ii*) proteolytic processing of the prolipoprotein to the final product of approximately half size. Two additional modifications will take place: N-acylation of the NH_2-terminal cysteine, and the joining of the free form lipoprotein to the murein sacculus through a peptide bond between the diaminopimelic acid and the ϵ-NH_2 of the COOH-terminal lysine.

The data presented in this paper strongly suggest that mutant 3 is altered in the structural gene for lipoprotein in such a way that the mutant lipoprotein is not processed normally. The resulting lipoprotein lacks the covalently-linked diglyceride, contains an unmodified cysteine and most interestingly, appears to be bigger in its apparent molecular weight than the wild-type lipoprotein. The incompletely processed lipoprotein found in this mutant is poorly assembled into the murein sacculi. These two novel biochemical features of the lipoprotein in mutant 3 (apparent increase in size and defective assembly into the murein sacculi) distinguishes this mutant from the *lpm*$^-$ mutant. This defect in the assembly of murein lipoprotein in mutant 3 may be related to its lipid deficiency. Alternatively, the defective assembly of mutant lipoprotein into the murein sacculi may be due to the presence of extra amino acids at either or both ends of this mutant lipoprotein, especially those at the COOH terminus. Preliminary evidence concerning the NH_2-terminal and COOH-terminal analysis as well as the amino acid composition of the mutant lipoprotein strongly supports the latter interpretation.

An alternative, albeit less likely, interpretation for the biochemical defect in mutant 3 is a defective processing enzyme. Because the mutations in both mutant 3 and *lpm*$^-$ mutant appear to be cotransducible with *aroD* (ref. 17; D. W. Yem and H. C. Wu, manuscript in preparation), they may represent different mutations in the structural gene for lipoprotein (*mlpA*) rather than in the same or different lipoprotein modifying enzymes (*lpm*). Final proof of this working hypothesis must await the determination of the primary structure of the lipoprotein in mutant 3.

The biochemical alterations found in mutant 3 lipoprotein could be demonstrated to be present in mutant cells and in the transductants grown at 30°, the temperature at which the mutant cells showed normal gross morphology, grew, and divided normally. It appears likely that neither a deficiency in the covalently-linked diglyceride of the lipoprotein nor a deficiency in murein-bound lipoprotein affects the growth, cell division, or morphology of the mutant cells. It remains to be ascertained whether the synthesis of free-form lipoprotein or prolipoprotein is essential for the viability of *E. coli* cells.

We are grateful to Dr. H. Suzuki and Dr. Yukinori Hirota for making strains JE5511 and JE5512 available to us. This investigation was supported by U.S. Public Health Service Grant CA-11371 from the National Cancer Institute, by Grant 75-632 from the American Heart Association, and by Grant BC-148A from the American Cancer Society.

1. Braun, V. & Rehn, K. (1969) *Eur. J. Biochem.* **10**, 426–438.
2. Inouye, M., Shaw, J. & Shen, C. (1972) *J. Biol. Chem.* **247**, 8154–8159.
3. Hirashima, A., Wu, H. C., Venkateswaran, P. S. & Inouye, M. (1973) *J. Biol. Chem.* **248**, 5654–5659.
4. Braun, V. & Sieglin, U. (1970) *Eur. J. Biochem.* **13**, 336–346.
5. Braun, V. & Bosch, V. (1972) *Eur. J. Biochem.* **28**, 51–69.
6. Hantke, K. & Braun, V. (1973) *Eur. J. Biochem.* **34**, 284–296.
7. Lin, J. J. C. & Wu, H. C. P. (1976) *J. Bacteriol.* **125**, 892–904.
8. Wu, H. C. & Lin, J. J. C. (1976) *J. Bacteriol.* **126**, 147–156.
9. Inouye, M. & Guthrie, J. P. (1964) *Proc. Natl. Acad. Sci. USA* **64**, 957–961.
10. Swank, R. T. & Munkres, K. D. (1971) *Anal. Biochem.* **39**, 462–477.
11. Hirs, C. H. W. (1967) in *Methods in Enzymology*, ed. Hirs, C. H. W. (Academic Press Inc., New York), Vol. 11, pp. 59–62.
12. Ozols, J. (1970) *J. Biol. Chem.* **245**, 4863–4874.
13. Inouye, S., Takeishi, K., Lee, N., DeMartini, M., Hirashima, A. & Inouye, M. (1976) *J. Bacteriol.* **127**, 555–563.
14. Moldow, C., Robertson, J. & Rothfield, L. (1972) *J. Membr. Biol.* **10**, 137–152.
15. Filip, C., Fletcher, G., Wulff, J. L. & Earhart, C. F. (1973) *J. Bacteriol.* **115**, 717–722.
16. Gurd, F. R. N. (1967) in *Methods in Enzymology*, ed. Hirs, C. H. W. (Academic Press Inc., New York), Vol. 11, pp. 532–541.
17. Suzuki, H., Nishimura, Y., Iketani, H., Campisi, J., Hirashima, A., Inouye, M. & Hirota, Y. (1976) *J. Bacteriol.* **127**, 1494–1501.
18. Halegoua, S., Hirashima, A. Inouye, M. (1976) *J. Bacteriol.* **126**, 183–191.