CHARACTERIZATION OF MUREIN-BOUND LIPOPROTEIN IN AN *ESCHERICHIA COLI* MUTANT ALTERED IN THE SIGNAL SEQUENCE OF PROLIPOPROTEIN

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1. Introduction

We have reported the isolation and characterization of an *Escherichia coli* mutant altered in the structural gene of murein lipoprotein (*mlpA*) [1,2]. The mutant lipoprotein differs from its wild-type counterpart in its total lack of covalently linked lipids and in its defective assembly into the murein sacculi of the mutant cell envelope [2]. The primary structure of the mutant lipoprotein has been determined [3]. It was found to correspond to an uncleaved precursor lipoprotein (prolipoprotein) with a single amino acid substitution of glycine by aspartic acid at position 14 of the prolipoprotein. As a consequence of this single amino acid replacement, all of the post-translational modifications of prolipoprotein are aborted.

The amount of murein-bound lipoprotein in the *mlpA* mutant is greatly reduced [1,2]. This biochemical alteration accounts for the phenotype of this mutant, i.e., increased EDTA sensitivity, leakage of the periplasmic enzyme RNase I, blebbing of the outer membrane upon Mg^{2+} starvation and greatly weakened outer membrane–murein association in *mlpAompA* double mutants [4]. Here we compare the structures of free and bound forms of lipoprotein in this mutant. Our results indicate that the murein-bound lipoprotein present in greatly reduced amounts is processed and modified by the addition of glycerol and fatty acids, while the bulk of free form lipoprotein in this mutant is uncleaved prolipoprotein.

2. Materials and methods

The *E. coli* strains used here were an isogenic pair of transductants E613 (*mlpA*$^+$) and E614 (*mlpA*) as in [4]. Media used in the present study included M9 minimal medium and proteose peptone beef extract broth [5].

All the labeling experiments were done in M9 minimal medium supplemented with 0.4% glucose or 1% sodium lactate and 50 μg of each required amino acid/ml. The preparation of cell envelope, identification of free-form lipoprotein with immunoprecipitation and isolation of murein-bound lipoprotein have been described [1]. Polyacrylamide gel electrophoresis in SDS [5] or SDS–urea [6] was used for the identification of lipoprotein.

Radioactive chemicals used in the present study included L-[3-^{3}H(*N*)]arginine (27 Ci/mmol), L-[U-^{14}C]arginine (270 mCi/mmol), L-[4,5-^{3}H(*N*)]-isoleucine (40 Ci/mmol), [2-^{3}H]glycerol (6.35 Ci/mmol) and [9,10-^{3}H(*N*)]palmitic acid (12.1 Ci/mmol) and were purchased from either New England Nuclear or Schwarz/Mann.

3. Results

3.1. *The amount of murein-bound lipoprotein in the* mlpA *mutant*

Amino acid composition of the murein sacculus isolated from the *mlpA* mutant revealed that the mutant cells contained ~10% of the wild-type level of bound-form lipoprotein (data not shown). Since the presence of contaminating and non-covalently linked proteins in the murein sacculus preparation may lead to an over-estimation, we have measured the amount of bound-form lipoprotein by a double-labeling experiment. [^{14}C]Arginine-labeled cell

envelope from the wild-type cells was mixed with >10-times the counts of [^{3}H]arginine-labeled cell envelope from the mutant cells. The murein sacculus was isolated from the mixed cell envelope, digested with lysozyme and analyzed by 10% SDS–polyacrylamide gel electrophoresis. The free-form lipoprotein was immunoprecipitated from the SDS-soluble envelope proteins with lipoprotein specific antisera and analyzed by 10% SDS–polyacrylamide gel electrophoresis. Assuming that the amounts of free-form lipoprotein are the same in the wild-type and the mutant cell envelope, the [^{3}H]/[^{14}C]ratio of the bound-form lipoprotein divided by the corresponding ratio of the free-form lipoprotein represents the relative amount of murein-bound lipoprotein in the *mlpA* mutant as compared to the wild-type cells. Based on this calculation, the amount of bound-form lipoprotein in the mutant cells is ~4% of that in the wild-type cells (table 1).

Table 1
Relative amount of murein lipoprotein in *mlpA* mutant E614 as compared to the wild-type strain E613

Fraction	[^{3}H]Arginine-labeled *mlpA* mutant / [^{14}C]Arginine-labeled wild-type
Total cell envelope	13.39
SDS-soluble envelope proteins	15.92
SDS-insoluble murein sacculi	1.11
Free-form lipoprotein	11.55
Bound-form lipoprotein	0.49

$$\frac{^3H/^{14}C \text{ of bound form}}{^3H/^{14}C \text{ of free form}} = \frac{\text{bound form/free form } (mlpA)}{\text{bound form/free form } (mlpA^+)} = \frac{0.49}{11.55} = 0.042$$

3.2. *The bound-form lipoprotein is processed*

While the free-form unprocessed lipoprotein of the mutant cells migrates faster than the wild-type lipoprotein in SDS gel and more slowly in SDS–urea gel, the bound-form lipoprotein of the mutant co-migrates with the wild-type bound-form lipoprotein. This result suggested that the much reduced bound-form lipoprotein in the mutant has already been processed.

Each prolipoprotein molecule contains 2 isoleucine and 4 arginine residues, whereas the mature lipoprotein molecule contains 1 isoleucine and 4 arginine residues

Table 2
Isoleucine/arginine ratio of the free-form and the bound-form lipoprotein in the *mlpA* mutant

Fraction	[^{3}H]Isoleucine / [^{14}C]Arginine
Free-form lipoprotein	1.46
Bound-form lipoprotein	0.74
Free-form lipoprotein[a]	1.28
Bound-form lipoprotein[a]	0.70

[a] Lipoproteins were immunoprecipitated with specific antisera and the immunoprecipitates were analyzed by SDS–urea polyacrylamide gel electrophoresis [6]

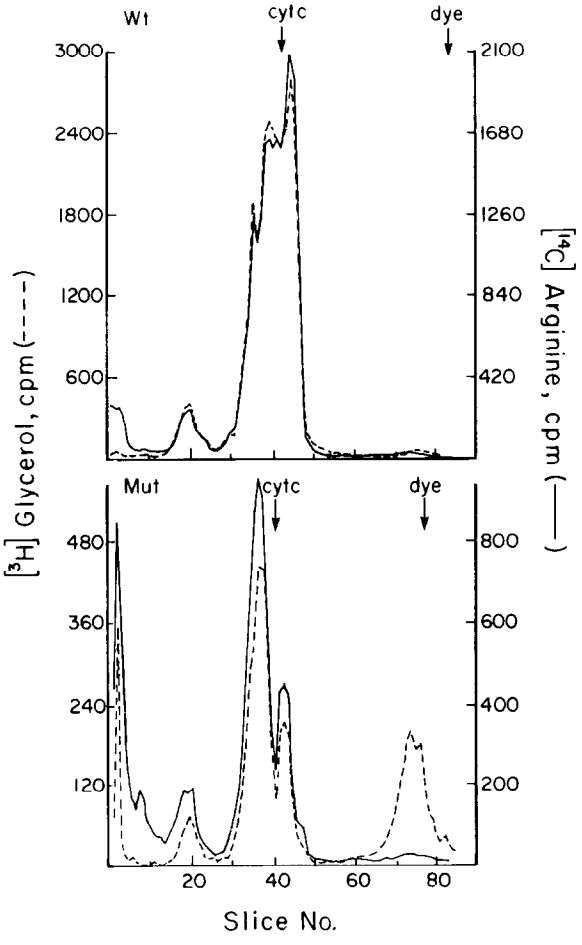

Fig.1. SDS–urea polyacrylamide gel electrophoresis of [2-^{3}H]glycerol- and [^{14}C]arginine-labeled murein-bound form lipoprotein from the wild-type (upper panel) and the *mlpA* mutant cells (lower panel).

[7,8]. The mutant cells were labeled with both [^{3}H]isoleucine and [^{14}C]arginine and the [^{3}H]/[^{14}C] ratios of both the free and bound forms of lipoprotein were determined. The results are shown in table 2. The [^{3}H]/[^{14}C]ratio of bound-form lipoprotein is 50% that of the free form. This result strongly suggests that the murein-bound lipoprotein in the *mlpA* mutant is already processed.

3.3. *The bound-form lipoprotein is modified by the addition of glycerol and fatty acids*

To ascertain whether the murein-bound form of lipoprotein in the *mlp*A mutant contains covalently linked lipids, we have labeled the wild-type and mutant cells with [2-^{3}H]glycerol and [^{14}C]arginine as well as with [^{3}H]palmitate and [^{14}C]arginine. Both the free and the bound forms of lipoprotein were analyzed by SDS–urea gel electrophoresis. As can be seen in fig.1,2, the bound-form lipoprotein in the *mlpA* mutant was labeled with both [2-^{3}H]glycerol and [^{3}H]palmitate to an extent similar to that found in the wild-type murein-bound lipoprotein. On the other hand, the free-form lipoprotein in the *mlpA* mutant exists mainly as the unmodified prolipoprotein which undergoes dimerization in the absence of 2-mercaptoethanol (fig.3,4). Alkali treatment (0.1 N NaOH for 2 h at 37°C) of [^{3}H]palmitate/[^{14}C]arginine labeled lipoproteins released 36%, 29% and 45% of [^{3}H]palmitate radioactivities from the wild-type free form, wild-type bound form and mutant bound form lipoproteins, respectively, and none from the mutant free form lipoprotein (data not shown). Total acid hydrolysis (6 N HCl for 20 h at 105°C) of both wild-type and mutant bound-form lipoproteins indicated that the [^{3}H]palmitate and [^{14}C]arginine radioactivities could be quantitatively recovered in chloroform layer and aqueous layer, respectively (data not shown). These results strongly suggest that the bound-form lipoprotein in the *mlpA* mutant contains both ester-linked and amide-linked fatty acids [9].

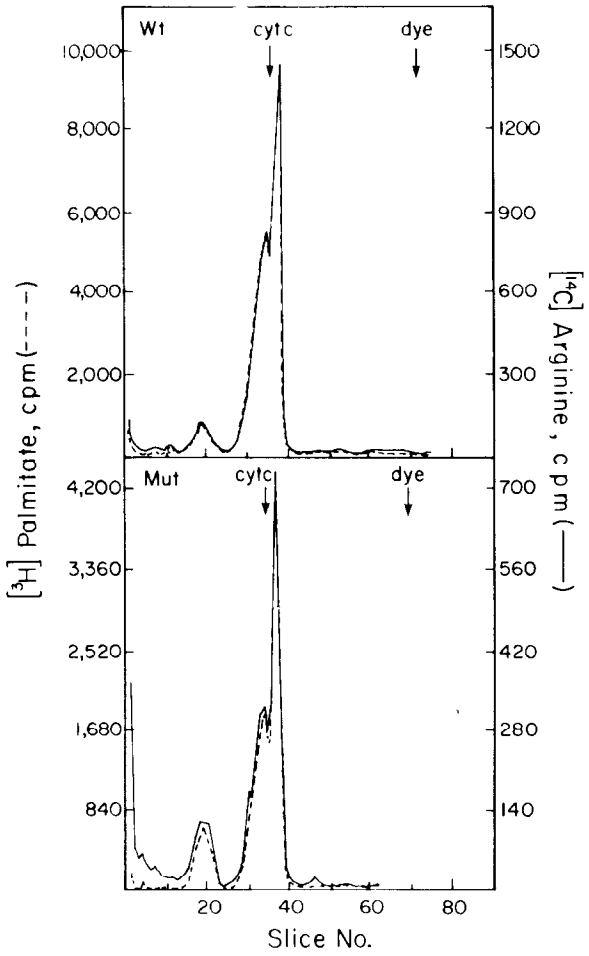

Fig.2. SDS–urea polyacrylamide gel electrophoresis of [^{3}H]palmitate- and [^{14}C]arginine-labeled murein-bound form lipoprotein from the wild type (upper panel) and the *mlpA* mutant cells (lower panel).

4. Discussion

We can conclude from these results that processing and post-translational modifications of the mutant prolipoprotein has occurred in the *mlpA* mutant to ~4% of that of the wild-type cells. Only the processed and fully modified lipoprotein is assembled into the murein sacculus, even though >50% of the uncleaved prolipoprotein is translocated to the outer membrane [3]. These results are consistent with the pathway for the biosynthesis of murein lipoprotein shown in fig.5. The assembly of lipoprotein into the murein sacculus requires a prior processing and proper modifications of the lipoprotein [9,10].

While the bound-form lipoprotein in the *mlpA* mutant is fully processed and modified, we fail to detect processed and modified free-form lipoprotein. This negative result might be simply due to the presence of a large excess of unprocessed prolipoprotein. Alternatively, this observation may suggest that the conversion of free-form lipoprotein to the murein-bound form may be irreversible.

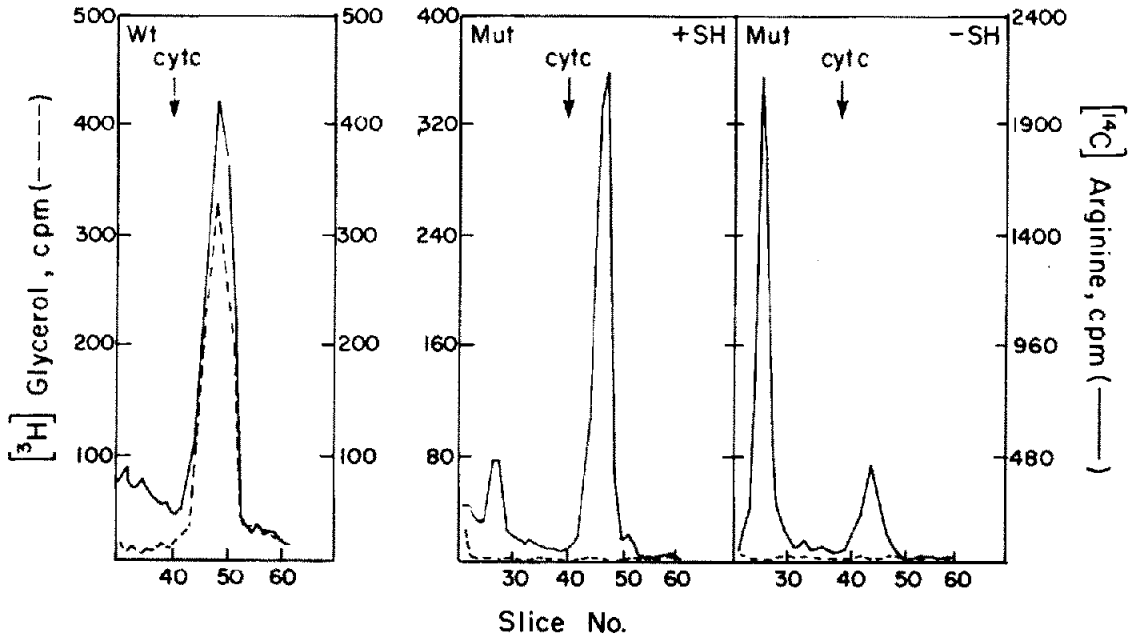

Fig.3. SDS–urea polyacrylamide gel electrophoresis of [2-^{3}H]glycerol- and [^{14}C]arginine-labeled free-form lipoprotein from the wild-type (left panel) and the *mlpA* mutant cells (right panels). Immunoprecipitates with lipoprotein specific antibodies were analyzed by SDS–urea gel electrophoresis in the presence (+SH) or absence (–SH) of 2-mercaptoethanol.

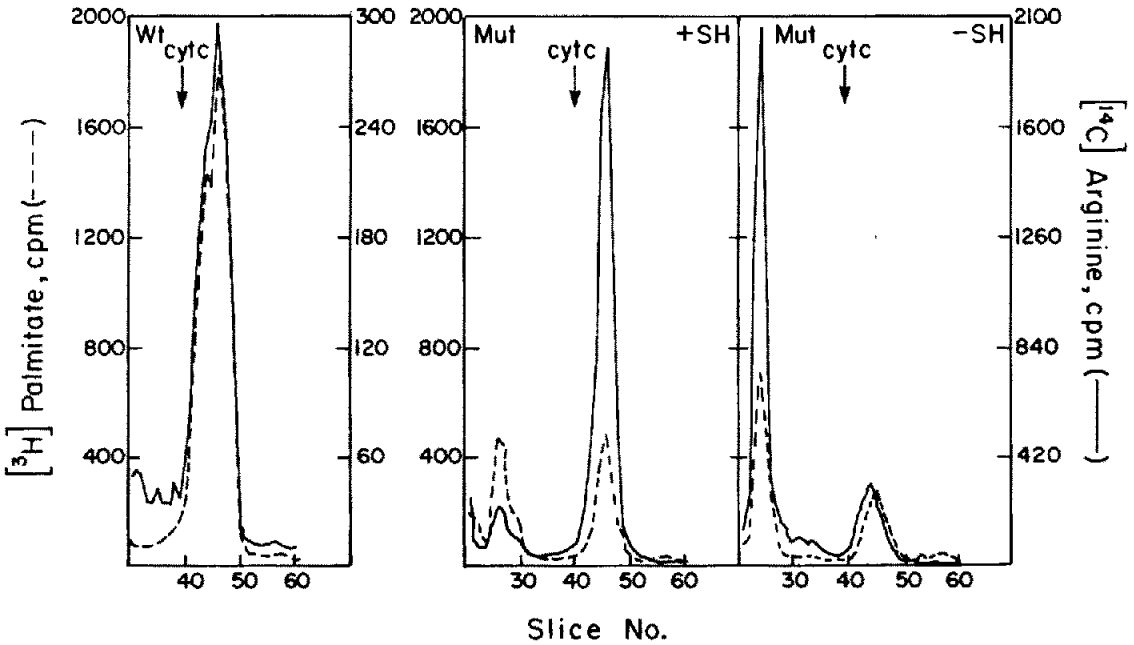

Fig.4. SDS–urea polyacrylamide gel electrophoresis of [^{3}H]palmitate- and [^{14}C]arginine-labeled free-form lipoprotein from the wild-type (left panel) and the *mlpA* mutant cells (right panels). Immunoprecipitates with lipoprotein specific antibodies were analyzed by SDS–urea gel electrophoresis in the presence (+SH) or absence (–SH) of 2-mercaptoethanol.

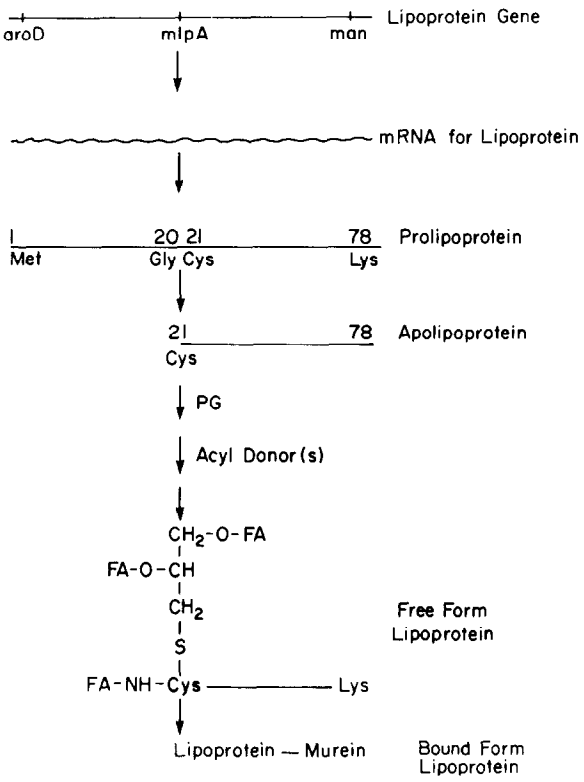

Fig.5. Postulated pathway of biosynthesis of murein lipoprotein in *E. coli*.

Acknowledgements

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References

[1] Wu, H. C. and Lin, J. J. C. (1976) J. Bacteriol. 126, 147–156.
[2] Wu, H. C., Hou, C., Lin, J. J. C. and Yem, D. W. (1977) Proc. Natl. Acad. Sci. USA 74, 1388–1392.
[3] Lin, J. J. C., Kanazawa, H., Ozols, J. and Wu, H. C. (1978) Proc. Natl. Acad. Sci. USA 75, 4891–4895.
[4] Yem, D. W. and Wu, H. C. (1978) J. Bacteriol. 133, 1419–1426.
[5] Inouye, M. and Guthrie, J. P. (1964) Proc. Natl. Acad. Sci. USA 64, 957–961.
[6] Swank, R. T. and Munkres, K. D. (1971) Anal. Biochem. 39, 462–477.
[7] Inouye, S., Wang, S., Sekizawa, J., Halegoua, S. and Inouye, M. (1977) Proc. Natl. Acad. Sci. USA 74, 1004–1008.
[8] Braun, V. and Bosch, V. (1972) Proc. Natl. Acad. Sci. USA 69, 970–974.
[9] Hantke, K. and Braun, V. (1973) Eur. J. Biochem. 34, 284–296.
[10] Chattopadhyay, P. K. and Wu, H. C. (1977) Proc. Natl. Acad. Sci. USA 74, 5318–5322.