

## N-acylation of lipoproteins: not when sour.

Nienke Buddelmeijer

► **To cite this version:**

Nienke Buddelmeijer. N-acylation of lipoproteins: not when sour.. Journal of Bacteriology, American Society for Microbiology, 2012, 194 (13), pp.3297-8. <10.1128/JB.00441-12>. <pasteur-01407694>

**HAL Id: pasteur-01407694**

**<https://hal-pasteur.archives-ouvertes.fr/pasteur-01407694>**

Submitted on 2 Dec 2016

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## N-Acylation of Lipoproteins: Not When Sour

Nienke Buddelmeijer

Institut Pasteur, Paris, France

Lipoproteins are characterized by the fatty-acylated amino termini by which they are anchored into biological membranes. This class of proteins is widespread among bacteria and represents approximately 2% to 3% of the proteome. They have a variety of biological functions, some of which are important for cell physiology and others for virulence. The lipid entity plays a crucial role, especially in bacterium-host interactions. For example, lipidated peptides are discriminated by host Toll-like receptors on the basis of the degree of fatty acid acylation: diacylated peptides interact with TLR2/6 heterodimers and triacylated peptides with TLR2/1 (5, 6). The classical lipoprotein modification pathway in species of *Proteobacteria* and *Actinobacteria* is composed of three sequential enzymatic reactions catalyzed by two acyltransferases and one signal peptidase. Phosphatidylglycerol:prolipoprotein diacylglyceryl transferase (Lgt) adds an *sn*-1,2-diacylglyceryl from phosphatidylglycerol to prolipoprotein, resulting in diacylglyceryl-prolipoprotein (10). Signal peptidase II (Lsp) then cleaves the signal peptide, resulting in an apolipoprotein containing a free  $\alpha$ -amino group. Apolipoprotein *N*-acyltransferase (Lnt) adds an *sn*-1-acyl chain derived from phosphatidylethanolamine (PtdEtn) to apolipoprotein, resulting in mature triacylated protein (3, 4). The genomes of *Firmicutes* and *Mollicutes* do not encode Lnt, and, with only Lgt and Lsp present, one might assume that these bacteria possess only diacylated lipoproteins with unmodified free  $\alpha$ -amino termini. Recent reports suggest, however, that *N*-acylation does occur in bacteria that lack an obvious *lnt* gene (1, 8, 9, 11). Kurokawa et al. recently showed that lipoproteins of low-GC Gram-positive bacteria have various modifications on their  $\alpha$ -amino groups (9). Not only *N*-acyl-*S*-diacyl but also *N*-acyl-*S*-monoacyl, *N*-acetyl-*S*-diacyl, and *N*-peptidyl-*S*-diacyl were detected, suggesting that *N*-acylation is a more widespread and diverse phenomenon than previously thought!

In this issue of the *Journal of Bacteriology*, the members of the Nakayama and Lee laboratories have published a report in which they present a solution to another longstanding issue involving *N*-acylation of lipoproteins in low-GC Gram-positive bacteria (7). Using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis, they demonstrated that environmental conditions affect the degree of fatty acid acylation such that lipoproteins can exist in different acylated states. They showed that lipoprotein SitC was triacylated when *Staphylococcus aureus* was in the exponential-growth phase at neutral pH and diacylated in the post-exponential phase at low pH. This result explains the apparently contradictory findings reported in two papers published in 2009 in which Tawaratsumida et al. demonstrated that one lipoprotein was diacylated whereas Kurokawa et al. showed that another protein was triacylated (8, 12). In their study, Kurokawa et al. showed that acidic pH is not sufficient for the accumulation of diacylated proteins and that protein synthesis is also required. They performed a pH downshift assay in the presence or absence of the protein synthesis inhibitor chloramphenicol and observed an increase in the diacyl form of

SitC only in the absence of chloramphenicol. At neutral pH, chloramphenicol prevented *N*-acylation. The authors concluded that *N*-acylation is most likely controlled by gene expression levels and not by the pH-dependent activity of the (as-yet-unidentified) Lnt. Jackowski and Rock showed in 1986 that, in a mutant of *Escherichia coli* lacking acyl-coenzyme A (acyl-CoA) synthetase (*fadD*) activity that selectively incorporates exogenous fatty acids into *sn*-1 of PtdEtn, protein synthesis was required for the turnover of the *sn*-1-acyl group. They demonstrated the direct involvement of Lnt in this process (4). So far, little is known about the regulation of the lipoprotein modification enzymes.

Stationary-growth-phase conditions and acidic pH could, however, affect *N*-acyltransferase activity directly. Bacteria are able to alter the fatty acid structure of their phospholipids upon changes in environmental conditions (13). Specific enzymes introduce *cis* or *trans* double bonds or cyclopropane rings in saturated fatty acids (2). Formation of the latter is activated upon entry into stationary phase. Since bacteria are unable to reverse cyclopropanation, the synthesis of saturated and *cis*-unsaturated fatty acids occurs during exponential growth. Lnt of *E. coli* has specificity for both the acyl chain length and composition of fatty acids; its preferred substrate is PtdEtn containing a saturated fatty acid (palmitate or C16:0) on *sn*-1 and an unsaturated fatty acid (oleic acid or C18:1) on *sn*-2 (3). The *sn*-1 acyl is transferred to the  $\alpha$ -amino group of apolipoprotein, but the *sn*-2 acyl moiety plays an important role (3, 4). One possibility is that, because the phospholipid substrate for Lnt changes during the stationary phase, lipoproteins can no longer be *N* acylated. Alternatively, other enzymes, such as deacylases, might be activated under specific growth conditions, such as acidic pH, that affect the ratio of diacyl and triacyl forms. Kurokawa et al. identified the diacyl structure of SitC by MALDI-tandem MS (MALDI-MS/MS) analysis and showed that *sn*-1 contained saturated fatty acids ranging from C17 to C20 and *sn*-2 contained saturated C15 lipids at acidic pH. Previous work by the same authors suggested that fatty acid moieties differ in degrees of saturation and acyl-chain lengths, depending on the protein and strain background (1). These findings suggest that the fatty acid structures of lipoproteins might also differ, depending on the environment.

These are exciting times for the bacterial lipoprotein field. Not only can we expect the identification of new modification enzymes but we will no doubt soon learn more about how the acylation

Published ahead of print 30 March 2012

Address correspondence to niebud@pasteur.fr.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.00441-12

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.

process is regulated, leading to new insights in lipoprotein function.

## REFERENCES

1. Asanuma M, et al. 2011. Structural evidence of  $\alpha$ -aminoacylated lipoproteins of *Staphylococcus aureus*. *FEBS J.* **278**:716–728.
2. Grogan DW, Cronan JE, Jr. 1997. Cyclopropane ring formation in membrane lipids of bacteria. *Microbiol. Mol. Biol. Rev.* **61**:429–441.
3. Hillmann F, Argentini M, Buddelmeijer N. 2011. Kinetics and phospholipid specificity of apolipoprotein N-acyltransferase. *J. Biol. Chem.* **286**:27936–27946.
4. Jackowski S, Rock CO. 1986. Transfer of fatty acids from the 1-position of phosphatidylethanolamine to the major outer membrane lipoprotein of *Escherichia coli*. *J. Biol. Chem.* **261**:11328–11333.
5. Jin MS, et al. 2007. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* **130**:1071–1082.
6. Kang JY, et al. 2009. Recognition of lipopeptide patterns by Toll-like receptor 2–Toll-like receptor 6 heterodimer. *Immunity* **31**:873–884.
7. Kurokawa K, et al. 2012. Environment-mediated accumulation of diacyl lipoproteins over their triacyl counterparts in *Staphylococcus aureus*. *J. Bacteriol.* **194**:3299–3306.
8. Kurokawa K, et al. 2009. The triacylated ATP binding cluster transporter substrate-binding lipoprotein of *Staphylococcus aureus* functions as a native ligand for Toll-like receptor 2. *J. Biol. Chem.* **284**:8406–8411.
9. Kurokawa K, et al. 2012. Novel bacterial lipoprotein structures conserved in low-GC content Gram-positive bacteria are recognized by Toll-like receptor 2. *J. Biol. Chem.* **287**:13170–13181.
10. Sankaran K, Wu HC. 1994. Lipid modification of bacterial prolipoprotein. Transfer of diacylglycerol moiety from phosphatidylglycerol. *J. Biol. Chem.* **269**:19701–19706.
11. Serebryakova MV, et al. 2011. The acylation state of surface lipoproteins of mollicute *Acholeplasma laidlawii*. *J. Biol. Chem.* **286**:22769–22776.
12. Tawaratsumida K, Fujimoto FMY, Fukase K, Suda Y, Hashimoto M. 2009. Characterization of N-terminal structure of TLR2-activating lipoprotein in *Staphylococcus aureus*. *J. Biol. Chem.* **284**:9147–9152.
13. Zhang YM, Rock CO. 2008. Membrane lipid homeostasis in bacteria. *Nat. Rev. Microbiol.* **6**:222–233.