Journal of the Korean Magnetic Resonance Society **2015**, *19*, 149-155
DOI 10.6564/JKMRS.2015.19.3.149

Effect of Acylation on the Structure of the Acyl Carrier Protein P

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Received Oct 03, 2015; Revised Nov 15, 2015; Accepted Nov 25, 2015

Abstract Acyl carrier protein is related with fatty acid biosynthesis in which specific enzymes are involved. Especially, acyl carrier protein (ACP) is the key component in the growing of fatty acid chain. ACP is the small, very acidic protein that covalently binds various intermediates of fatty acyl chain. Acylation of ACP is mediated by holo-acyl carrier protein synthase (ACPS), which transfers the 4'PP-moiety of CoA to the 36th residue Ser of apo ACP. Acyl carrier protein P (ACPP) is one of ACPs from *Helicobacter plyori.* The NMR structure of ACPP consists of four helices, which were reported previously. Here we show how acylation of ACPP can affect the overall structure of ACPP and figured out the contact surface of ACPP to acyl chain attached during expression of ACPP in *E. coli*. Based on the chemical shift perturbation data, the acylation of ACCP seems to affect the conformation of the long loop connecting helix I and helix II as well as the second short loop connecting helix II and helix III. The significant chemical shift change of Ile 54 upon acylation supports the contact of acyl chain and the second loop.

Keywords Acyl carrier protein, ACPP, Acylation, fatty acid biosynthesis, Structure, NMR

Introduction

Acyl carrier protein is related with fatty acid biosynthesis in which specific enzymes are involved.[1-3] The enzyme systems of fatty acid biosynthesis are quite different between bacteria and animal.[4,5] In bacteria, fatty acid synthase II (FASII) plays a pivotal role in the biosynthesis of fatty acids whereas the synthesis is mainly mediated by fatty acid synthase I (FASI).[6] The family of FASII is characterized by the use of discrete, monofunctional enzymes for the systhesis. The similarity of FASII enzymes between bacteria is usually conserved so that the working mechanism of the enzymes is almost same. Absence of the FAS-related proteins are usually lethal for growth and survival of bacteria such *E. coli, B. subtilis*, and so on since they participate in building of the cell envelope. In the various fatty acid biosynthesis, the fatty acid chain elongation is catalyzed by separate monfuntional synthases.[7-9]

Especially, acyl carrier protein (ACP) is the key component in the growing of fatty acid chain. ACP is the small, very acidic protein that covalently binds various intermediates of fatty acyl chain produced in the synthesis of fatty acids. ACP exists in either an acylated or apo form (free of acyl-chain) in which acylation of ACP is mediated by holo-acyl carrier protein synthase (ACPS), which transfers the 4'PP-moiety of CoA to the 36th residue Ser of apo ACP.[10-13] The structures of ACPs have been largely determined by NMR of X-ray crystallography, which suggested that the structures are highly conserved in many bacterial strains; they adopt a four α-helical

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(A)

	10	20	30	40	50	60	70
ACPP	MALFEDIQA VIAEQLNVDA VQVTPEAEFV KDLGAD	SLDV VELIMALEEK FG	VEIP	DEQA E	KIINV	GDVV KYIEDNK	LA

(B)

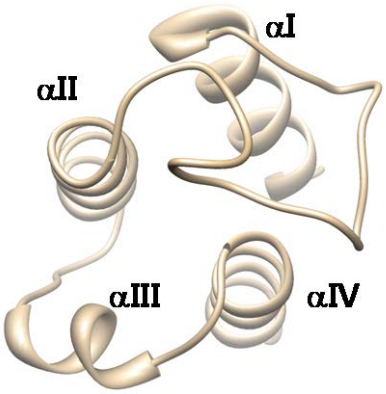

Figure 1. The structure of ACPP[24]. (A) The secondary structure of ACPP is depicted on the amino acid sequence of ACPP. (B) The four helices are represented with the symbol, α.

bundle which are connected by long loops of various lengths. The three helices I, II, and IV are arranged to form an up-down-down topology and the third helix III usually locates almost perpendicular to the above three helix bundle.[14-23] Though the binding modes between ACP and ACPS seem to be divergent in various enzymes, helices II and III of ACP is likely to play central role in complex formation between two proteins. In *E. coli*, the complexation of acylated-ACP with ACPS is predominantly mediated by the helix I of ACPS and the helix III of ACP. The interaction is hydrophobically regulated by Leu 37 and Met 44 in the helix II of ACP.

Acyl carrier protein P (ACPP) is one of ACPs from *Helicobacter plyori.* The ORF of ACPP is HP0559 and the protein is composed of 78 amino acids. Previously, we showed the NMR structure of ACPP (Figure 1); [24] it consists of four helices corresponding to residues L3-Q14 (αI), S36-G50 (αII), D56-E60 (αIII), and V65-K76 (αVI). The structural characteristics of ACPP were very similar to those of other ACPs while we suggested usual stability of the structure depending on pH;[24] the destabilization of the ordered structure in the neutral pH occurs by conformational change of helices II and III with rearrangement of loop II, accompanied by loss of rigidity of loop I region in the vicinity of helix II. Actually, the disruption of interaction between several hydrophilic residues that locate in the hydrophobic core of the helical bundle was monitored in the altered pH condition.

Here we show how acylation of ACPP can affect the overall structure of ACPP and figured out the contact surface of ACPP to acyl chain attached during expression of ACPP in *E. coli*.

Experimental Methods

ACP Sample Preparation- The subcloning and purification were described elsewhere in detail. Briefly, The ORF of ACPP (HP0559, 78 a.a) was amplified using PCR and inserted into a pET 21a vector. The pfu DNA polymerase were used for production of target DNA with two overlapping primers, 5'-GGAATTCCATATGGCTTTATTTGAAGATAT TCAGGC-3'and5'-CCGCCGCTCGAGTGAAGCC AGTTTATTATCCTCAATATACTT-3' containing the restriction enzyme sites for NdeI and XhoI, rescpectively.

The uniform ^{15}N- labeled ACPP was expressed in *E. coli* BL21(DE3). The purification was conducted as follows; the cell pellet was resuspended in the lysis buffer consisting of 50 mM Tris-Cl and 500 mM NaCl (pH 8.0). After lysis by sonication, the clear supernatant was loaded onto Ni-chelating resin (Amersham Pharmacia Biotech). ACPP was eluted with the elution buffer containing 300 mM immidazole. The eluted solution was dialyzed overnight against 50 mM Na.phosphate (pH 7.0), 10 mM NaCl, 1 mM EDTA, and 1 mM DTT and loaded on a DEAE column (Amersham Pharmacia Biotech). ACPP was obtained by elution with NaCl gradient. More purification was done with a size exclusion chroma-

tography prior to the NMR measurement. ACPP that expressed in *E.coli* usually form acylated structure with a variety of saturated long-chain fatty acids. The structural information of the acylated ACPP was obtained from this sample. To get apoACPP, the purified protein was treated with 100 mM DTT at 37 ℃ overnight to remove acyl chains. The insoluble acyl-DTT was formed during the reaction and the resultant precipitation was removed by centrifugation.

NMR spectroscopy- NMR spectra were acquired on a Bruker AVANCE 600 MHz spectrometer at 303 K. The NMR measurement was done with ^{15}N-labelled ACPP. 2D ^{15}N HSQC specta (Bodenhausen et al., 1980) were obtained for both the acylated and apo forms. Unless indicated otherwise, all ACPP samples were dissolved in 90% H_2O/10% D_2O, containing 50 mM potassium phosphate and 1 mM EDTA (pH 6.0). The concentration of the NMR sample ranged between 2 and 4 mM. All heteronuclear NMR spectra were recorded with gradient sensitivity enhancement in the first indirect heteronuclear dimension of the 2D and 3D experiment. Indirect dimensions were processed after appropriate rearrangements of FIDs according to the States-TPPI procedure. Chemical shift calibrations for all nuclei were performed relative to the proton resonance of DSS as suggested previously.[25] Spectra were processed with NMRPipe[26] and analysed with NMRView 5.

Secondary structure predictions were made from an analysis of the ^{13}Cα, ^{13}Cβ, and ^{13}CO chemical shifts using the CSI program of Wishart and Sykes.[27]

The ^{1}H and ^{15}N chemical shifts of the acylated form were compared with those of the apo form. The unit of Y axis is ppm and X axis is residue number. The weight-averaged chemical shift differences was calculated for each residues using following equation, $\Delta_{avg}(HN) = \{((\Delta H)^2 + (\Delta N/5)^2)/2\}^{1/2}$.

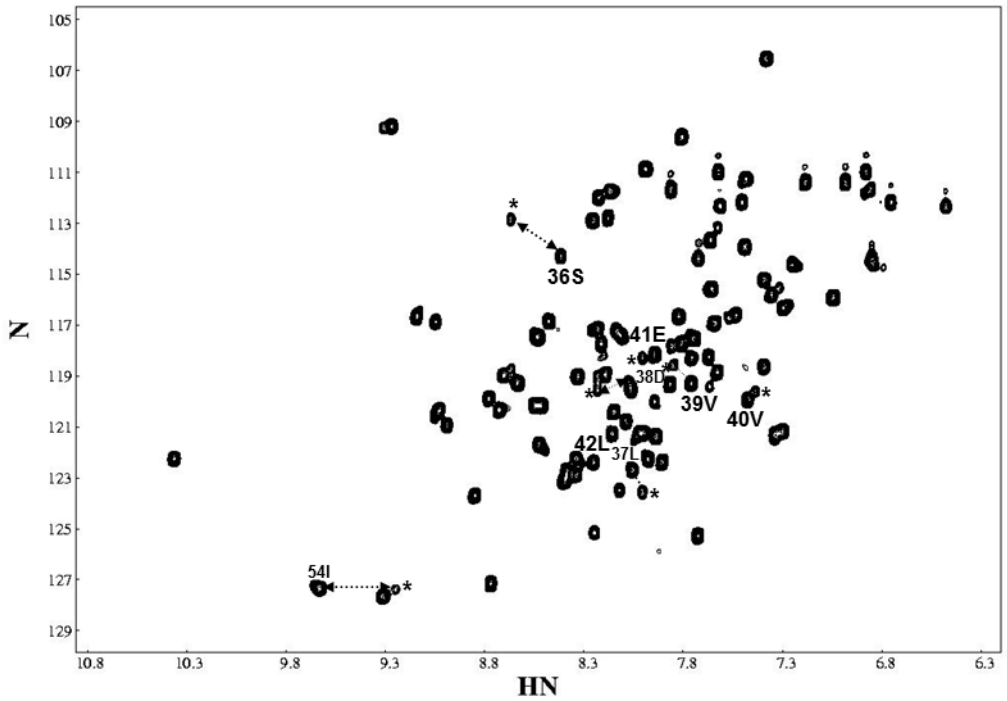

Figure 2. Representative residues affected by acylation of ACPP. Some population of *E. coli*-expressed ACPP possesses the acyl chains intrinsically. The conformers between apo- and acylated-ACPP can be identified in the HSQC spectrum. The asterisks represent the shifts by acylation.

Results and Discussion

In our previous study, 3D-structure of ACPP was determined in 500 mM NaCl, 50 mM potassium phosphate, 1 mM DTT, and 1 mM EDTA, at pH 6.0 and the result showed that ACPP has the four α-helical bundle structure with a hinge region in between helix I and helix II.[9] To investigate the effect of attachment of fatty acyl chain to the residue Ser 36, the chemical shifts of apo-ACPP and acylated-ACPP were compared using HSQC spectra. The information of acylated-ACPP can be easily identified using the purified protein since the the *E. coli*-expressed ACPP possesses already the acyl chain covalently bonded to Ser 36. The usage of high concentration of the reducing agent DTT breaks this covalent bond, resulting in easy obtaining of apo-ACPP. Figure 2 clearly shows some portion of NMR sample contains the acylated-ACPP. The shifted peaks by acylation are depicted with the asterisks. The residue Ser 36, as expected, shows the significant shift.

Resonance assignments for Acylated-ACPP- We performed sequence-specific resonance assignments for acylated-ACPP using mainly HNCA, HNCACB, and NOESY-HSQC data, referencing by 4,4-dimethyl-4-silapentane-1-sulfonate. The sequential assignments for acylated form were almost completed up to 98% for the backbone amides and protons. The overall chemical shifts of acylated form were very similar to those of apo form except several residues. Sequential NOE connectivities and other NMR data for connection are not shown here.

Chemical shift perturbation by acylation- To compare the chemical shifts between two forms in detail, we calculated weighted-average chemical shift changes based on the equation described in the materials and methods. The resultant difference plot along with the residue numbers is shown in the figure 3. The pattern of weighted-average chemical shift difference is similar to that of backbone amide proton chemical shifts and backbone amide nitrogen chemical shifts.

The average of weighted-average chemical shift changes for all residues was 0.0145 ppm, suggesting the structural difference between two forms may be not so significant. However, we could see several notable changes such as Ala 34, Ser 36, Leu 37, Asp 38, Glu 41, Met 44, Leu 46, Glu 47, Lys 49, Glu 53, Ile 54, Val 69, Asp 74, Asn 75, Lys 76, Leu 77, and

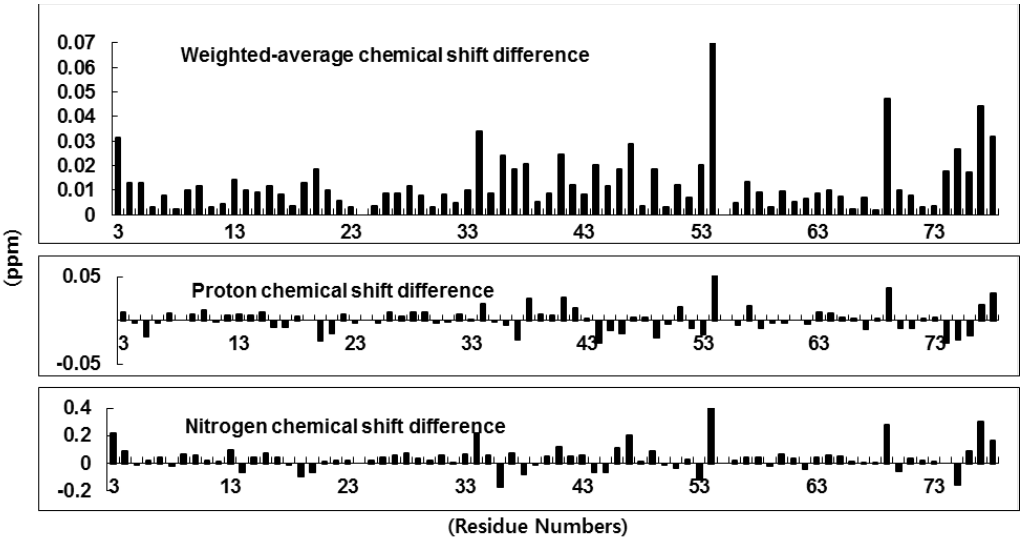

Figure 3. Weighted-average chemical shift changes of backbone amide proton and nitrogen induced by acylation. The X-axis is the residue numbers of ACPP and the Y-axis is chemical shift ppm for each plot. (Top) The change of weighted-average of proton and nitrogen (Middle) chemical shift perturbation of backbone amide proton by acylation (Down) chemical shift perturbation of backbone amide nitrogen by acylation.

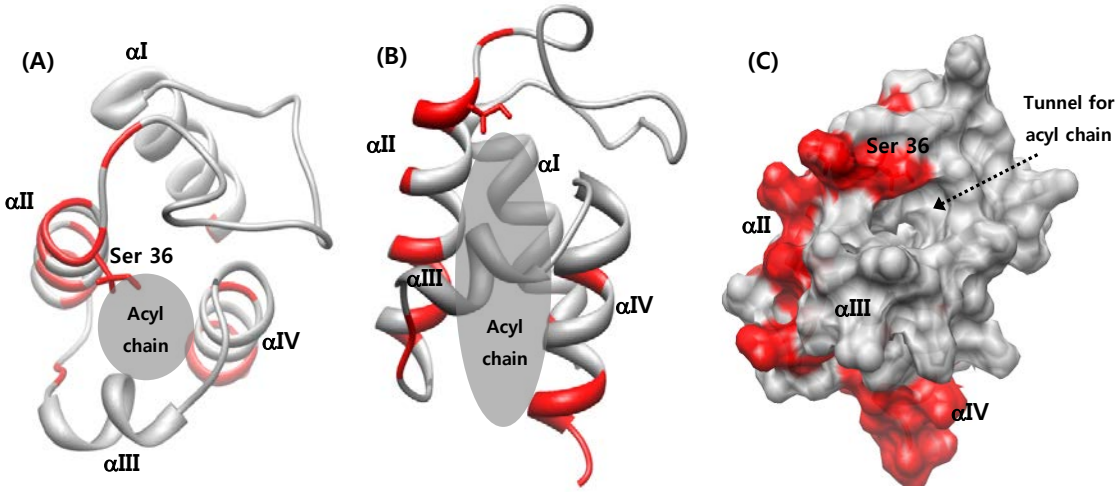

Figure 4. Perturbed chemical shifts mapping induced by acylation of ACPP. The structure of apo-ACPP used for illustration was the structure published elsewhere. **(A)** Ribbon representation of apo-ACPP. The red colored residues are the most significantly perturbed residues by acylation. The symbol, α represents a helix. The side-chain of acylation site, ser 36 is depicted. The gray circle represents the location of acyl chain, which was estimated by the perturbed changes shown in Figure 3. **(B)** Side view of perturbed region **(C)** Surface representation of (B). A deep tunnel exists on the structure of ACPP.

Ala 78.

Mapping of perturbation by acylation on the structure of apo-ACPP- To understand the conformational change induced by acyl-chain attachment, we analyzed the chemical shift changes with the 3D structure. The significantly perturbed resonances were mapped on the structure of apo-ACPP with red color (Figure 4).

The most affected regions correspond to the long loop 1 containing Ser 36, helix 2, the second loop containing Ile 54, and the terminal region of helix 4. Interestingly, the interface of acyl chain and ACPP seems to locate the room between two helices, helix 2 and helix 3 as shown in figure 4 (A). In the side view point like figure 4 (B), the acyl chain seems to be elongated from top of helix 2 through covalently bonding to side-chain of Ser 36 into the bottom of helix 4. This space is actually the hydrophobic core of ACPP maintained by hydrophobic network between helix 1, helix 2, and helix 3. This result may suggest that acylation by ACPS (acyl carrier protein synthase) may not occur on the surface of ACPP and large conformational changes should be followed during acylation process. How can a long acyl chain be transferred into the hydrophobic core of ACPP? We could suspect a gate that is related with entrance and exit of acyl chain. Notably, the surface representation of ACPP showed a long and deep tunnel around the residue Ser 36 as shown in figure 4 (C). The widths of top and bottom and left and right is approximately 6.2 Å and 9.89 Å, respectively and

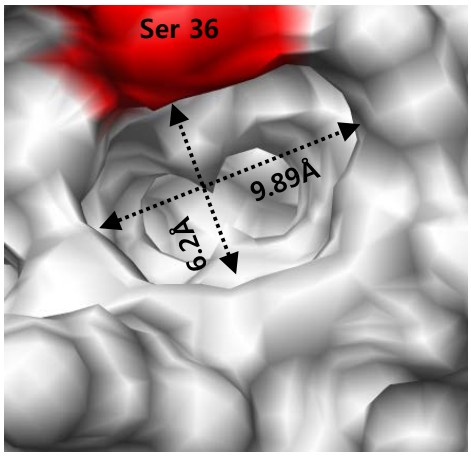

Figure 5. The long and deep tunnel exists around the long loop 1 near Ser 36. The two widths of the entrance of the tunnel are depicted on the structure.

the depth of the tunnel is around 17.75 Å (Figure 5). Considering one of the fatty acids, the stearic acid consisting of 18 carbon chain is around 13-14 Å in length, the depth of the tunnel is not insufficient for entering of several acyl chains. As a result, this tunnel may be related with the acyl chain trafficking mediated by ACPS and ACPP. Although the opening and closing mechanism is still questionable, the existence of the tunnel may be helpful to understand the elongation of acyl chain.

In this study, we examined the effect of acylation of ACPP using NMR. A Recent report showed that the 1.9 Å crystal structure of the crosslinked AcpP–FabA complex as a homodimer.XX FabA is the β-hydroxydecanoyl-ACP dehydrase that interact with β-hydroxydecanoyl-ACPP.[29] The report showed clear complex structure between two proteins and they proposed the acyl chain delivery takes place on the surface of two proteins. However, the proposed mechanism could not completely explain the existence of the tunnel for acyl chain trafficking and more studies are required for understanding.

Acknowledgements

This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (NRF-2014M3A9B6069340). This work was also supported by grants from the Basic Science Research Program (2013R1A1A2064418) through the National Research Foundation of Korea, funded by the Ministry of Education, Science, and Technology.

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