

A novel approach for over-expression, characterization, and isotopic enrichment of a homogeneous species of acyl carrier protein from *Plasmodium falciparum*

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Abstract

Acyl carrier protein (ACP) plays a central role in fatty acid biosynthesis by transferring the acyl groups from one enzyme to another for the completion of the fatty acid synthesis cycle. Holo-ACP is the obligatory substrate for the synthesis of acyl-ACPs which act as the carrier and donor for various metabolic reactions. Despite its interactions with numerous proteins in the cell, its mode of interaction is poorly understood. Here, we report the over-expression of PfACP in minimal medium solely in its holo form and in high yield. Expression in minimal media provides a means to isotopically label PfACP for high resolution multi-nuclear and multi-dimensional NMR studies. Indeed, the proton–nitrogen correlated NMR spectrum exhibits very high chemical shift dispersion and resolution. We also show that holo-PfACP thus expressed is amenable to acylation reactions using *Escherichia coli* acyl-ACP synthetase as well as by standard chemical methods.

Keywords: Protein expression; *Plasmodium falciparum*; Apo-ACP; Holo-ACP; Acyl-ACPs; NMR

Fatty acid biosynthesis is organized in two distinct architectural units in living organisms. Type I or associative type is present in metazoans and is characterized by a single large polypeptide chain with multiple domains for the completion of fatty acid synthesis [1]. In contrast, bacteria, plants, and certain protozoans synthesize fatty acids by type II or dissociative type, where all the individual reactions are carried out by discrete enzymes [2]. Recently, we have shown that *Plasmodium falciparum* synthesizes fatty acids utilizing type II fatty acid synthase (FAS) [3]. Because of its fundamental differences from the type I FAS present in the human host,

this pathway is an attractive target for the development of potent antimalarials [3,4].

Acyl carrier protein is an essential component of FAS, which plays a central role in fatty acid synthesis, be it the type I or type II system [5]. In type I FAS, ACP is an integral part of the multi-enzyme complex and the nascent fatty acyl chain swings between active sites of successive enzymes, whereas in type II FAS, acyl carrier protein is a separate entity, which takes the growing acyl chain from one enzyme to another for the completion of synthesis [1,2,5]. Apart from fatty acid biosynthesis, ACP plays a key role in many other reactions that require acyl transfer, such as synthesis of polyketides [6], phospholipids [7], lipid A [8], lipoic acids [9], biotin [10], quorum sensing molecules such as acylated homoserine lactones [11], hemolysin [12], rhizobial nodulation signaling factors,

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etc. [13]. ACP is a small, acidic, and highly conserved protein. Most of its residues at the prosthetic group attachment site and at the central helix, helix $\alpha 2$, are conserved throughout the living systems [14]. In vivo ACP is initially expressed as an inactive form, apo-ACP, which is converted to active holo form, by attachment of 4'-phosphopantetheine group of coenzyme A (CoA) to the β -hydroxyl side chain of its conserved central serine residue. The post-translational modification of apo-ACP to holo-ACP is catalyzed by holo-ACP synthase (ACPS) [15]. The growing acyl chain is covalently attached by a thioester bond to the terminal sulfhydryl of 4'-phosphopantetheine prosthetic group and is shuttled from one enzyme to another in the above-mentioned pathways [5,15,16]. While acyl-ACPs are devoid of any catalytic activity, they are obligatory substrates for all acyl group transfer reactions. All of these reactions occur with great precision and specificity without invoking extensive protein-protein interaction [16]. Despite its recognition by many enzymes, no single motif of ACP for these interactions has yet been identified, though it has been suggested that ACP interacts with them via conserved electrostatic and hydrophobic contacts mainly through helix II [14,17].

For the biochemical and structural characterization of the various PfACP forms and their enzyme complexes by nuclear magnetic resonance (NMR) and X-ray crystallography, pure holo-PfACP/acyl-ACPs are required. In vivo, ACP is mainly found in holo-form but its over-expression in a heterologous host such as *Escherichia coli* gives rise to all the three forms; apo-, holo-, and acyl-ACPs, entailing several chromatographic steps to obtain a given form from the mixture [18–23]. In this article, we report a simple and reproducible procedure for the over-expression of holo-PfACP as a hexa-histidine-tagged protein. The circular dichroism (CD) spectra and 1D proton NMR of PfACP are being reported. We also show that the over-expressed holo-PfACP using this method is properly folded and is biologically active.

Materials and methods

Luria broth was purchased from Difco. Kanamycin, isopropyl- β -D-thiogalactopyranoside (IPTG), and thrombin were from Sigma-Aldrich, octanoic acid, lauric acid, and myristic acid were obtained from Merck. The restriction enzymes were from MBI Fermentas, pGEM-T Easy vector system I and T4 DNA ligase were from Promega. Ni-NTA resin was purchased from Novagen. $^{15}\text{NH}_4\text{Cl}$ and ^{13}C glucose were supplied by Cambridge Isotopes USA. All other chemicals used were of analytical grade.

Strains and vectors

Escherichia coli DH5- α cells were from Gibco-BRL, *E. coli* BL21-(DE3) cells and pET-28a(+) vector were from Novagen whereas C41 (DE3) cells were from Avidis (France).

Cloning of *P. falciparum* acyl carrier protein

Maintenance of *P. falciparum* culture. Clone FCK-2 (chloroquine-sensitive, IC_{50} , 18 nM), an isolate of *P. falciparum* from Karnataka, India, was maintained in continuous culture. Briefly, erythrocytic stage parasites were cultured in RPMI 1640 supplemented with 10% human serum, 25 mM sodium bicarbonate, and 25 mM Hepes at a 10% hematocrit, according to the method of Trager and Jensen [24].

Cloning of PfACP in *E. coli*. Total RNA was isolated from *P. falciparum* culture [25] and cDNA was synthesized using reverse transcription kit (MBI Fermentas) and PfACP specific reverse primers. The following primers were designed for PCR using GenBank Accession No. AF038928, to clone the mature protein (without the leader peptide and transit sequence, required for targeting the protein to apicoplast):

Forward primer: PfACPmat-F 5'-ggAATTCCATATg TTA AAA AgT ACT TTT gAT gAT ATT AAA AAA ATT ATA TCA AAg C-3'

Reverse primer: PfACPmat-R 5'-CgggATCC TTA TTg CTT ATT ATT TTT TTC TAT ATA ATC TAT AgC-3' (with *NdeI* and *BamHI* sites underlined, respectively)

PCR was carried out using the following conditions: denaturation -94°C , 5 min for the first cycle, 94°C , 1 min for remaining 23 cycles, primer annealing -57°C , 30 s, and extension -72°C , 1 min followed by extension -72°C for 7 min. The 237-bp RT-PCR product was excised from 1.2% agarose gel, purified using AuPrep gel extraction kit (Life Technologies, India), and cloned in pGEM-T Easy vector (Promega) in *HindIII* and *NdeI* sites. The insert in pGEM-T Easy clone was digested with *NdeI* and *BamHI*, and subcloned in pET-28a (+) vector (Novagen) in-frame with the N terminus 6-His tag and the sequence was confirmed by DNA sequencing (Microsynth GmbH, Switzerland). Over-expression was carried out using BL-21[DE3] cells.

Over-expression of *P. falciparum* acyl carrier protein. A single colony was inoculated in 20 ml Luria broth and was grown overnight at 37°C with vigorous shaking. M9 minimum medium was then inoculated with 1% primary culture and grown at 37°C , 200 rpm, until A_{600} reached 1.0. The cells were then induced with 0.4 mM IPTG and grown at 25°C for 9 h, harvested at 4000 rpm, and stored at -80°C until further use.

Purification

All the purification steps were carried out at 4°C unless otherwise indicated. Cells were thawed at room temperature in 20 mM Tris, 200 mM NaCl, and 10 mM imidazole-HCl, pH 7.4 (resuspension buffer), lysed using French press at 1100 psi, and centrifuged for 60 min at 15,000 rpm. The clear supernatant was loaded on Ni-NTA column. The column was washed with 20 column volumes of resuspension buffer and 10 column volumes of the same buffer containing 25 mM imidazole, pH 7.4. *P. falciparum* acyl carrier protein (PfACP) was eluted with resuspension buffer containing 100 mM imidazole. The purity of the protein was checked on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Analysis of holo- and apo-forms of PfACP

To check the presence of holo- and apo-PfACP, 13% native polyacrylamide gel electrophoresis (PAGE) containing 2.5 M urea was run at 20 mA [26]. The gel was stained with Coomassie blue and destained according to the standard protocol. Protein was estimated by the Bradford method [27] and also by spectrophotometric method using the extinction coefficient of PfACP ($1280 \text{ M}^{-1} \text{ cm}^{-1}$ at 280 nm).

Extinction coefficient of his-tagged PfACP and the PfACP devoid of the tag was calculated using Prot Param tool available in Swissprot public domain [<http://tw.expasy.org/tools/protparam.html>].

Removal of His-tag from holo-PfACP

The purified holo-PfACP was dialyzed overnight in 140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 1.8 mM KH₂PO₄, pH 7.3, with two changes. Thrombin (Sigma) was dissolved in 1× thrombin cleavage buffer to a concentration of 1 U/μl. Holo-PfACP was incubated with thrombin (1 U/mg) for 2 h at 22 °C, and the reaction was stopped by the addition of 1 mM phenylmethylsulfonyl fluoride (PMSF). The cleaved holo-PfACP was recovered by loading the mixture on Ni-NTA column equilibrated with 20 mM Tris-HCl, 200 mM NaCl containing 10% glycerol (pH 7.4), and was stored in -20 °C at a concentration of 0.5 mM for further use.

Mass spectrometry of holo-PfACP

The samples were dialyzed against water for 12 h and mixed uniformly with 1 μl of the matrix, prepared by adding 0.05% TFA (trifluoroacetic acid) to a saturated solution of sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid), and spotted onto the MALDI plate. After the spot dried completely, it was excited with laser shots and the mass of the various forms of PfACP was determined using MALDI mass spectrometry, on the Ultra Flex TOF/MALDI-TOF mass spectrometer from Bruker Daltonics

Circular dichroism studies of holo-PfACP

Circular dichroism (CD) spectra were recorded on a Jasco J-715 spectropolarimeter in a quartz cuvette (1 mm path length) at a protein concentration of 10 μM in 10 mM Tris, 100 mM NaCl (pH 7.4) buffer.

Synthesis of acyl-PfACP substrate

Acyl-PfACPs were synthesized by chemical as well as enzymatic methods [28,29]. Acyl-ACP synthetase (AAS) of *E. coli* was over-expressed in C41 [DE3] cells and purified as described earlier except that the hexa-histidine tag was at the N-terminus [30]. In brief, the enzymatic acylation reaction contained 5 mM ATP, 10 mM MgCl₂, 0.4 M LiCl, 2 mM DTT, 2% Triton X-100, 160 mM fatty acids, 10 mg PfACP, and 1 mg of freshly prepared acyl-ACP synthetase (AAS) from *E. coli* (in 100 mM Tris-HCl buffer, pH 8). The reaction mixture (10 ml) was initially kept at 37 °C for 3 h and shifted to 25 °C for 12 h. The reaction was stopped by adding three volumes of 100 mM Tris-HCl, pH 8. The product was purified using octyl-Sepharose as described in [28]. The acyl-ACPs thus synthesized were checked by conformational sensitive PAGE and confirmed by MALDI.

Preparation of ¹⁵N and ¹³C labeled holo-PfACP for NMR studies

Isotopically enriched ¹⁵N and ¹⁵N/¹³C labeled PfACP samples were prepared by growing PfACP construct in M9 minimal medium, containing labeled ¹⁵NH₄Cl (1 g/L) and [¹³C]glucose (2 g/L) in place of natural analogs as the sole source of nitrogen and carbon, respectively. The labeled holo-PfACP was purified by the same method used for its unlabeled counterpart.

NMR data acquisition

Samples for NMR spectroscopy were prepared by dialyzing holo-PfACP against 50 mM phosphate buffer, pH 6.5, containing 100 mM NaCl, 0.5% NaN₃, 2 mM DTT in 90% H₂O/10% D₂O mixture, and 1 mM DSS (sodium 4,4-dimethyl-4-silapentanesulfonate) as internal

standard. All NMR spectra were recorded with ¹⁵N, ¹³C doubly enriched holo-PfACP sample of 1.4 mM concentration on a Bruker Avance 700 MHz spectrometer equipped with 5 mm cryoprobe and *z* axis pulse field gradient. The sample temperature was maintained at 300 K throughout the experiment. One-dimensional NMR spectrum was acquired using excitation sculpting pulse sequence [31]. All proton chemical shift values were in parts per million and were referenced with respect to internal DSS. The three-dimensional HNCACB [32,33] data set was acquired as a 2048 * 32 * 128 complex data matrix. All NMR data were processed on an Intel PC workstation running on Suse Linux 8.2 using NMRPipe/NMRDraw processing software [34]. A Gaussian filter with a line-broadening parameter of 15 Hz was applied in direct and indirect acquired dimensions. Data sets were zero-filled once in each dimension to yield a final matrix of 1024 * 64 * 256 real data points. NMR data were analyzed using ANSIG [35].

Results and discussion

It has been shown that type II fatty acid synthesis takes place in *P. falciparum*. [3,22,36–39]. This finding has given tremendous impetus to the search for the development of new antimalarials targeting the proteins of FAS [3,4,38]. In *P. falciparum* the gene coding for acyl carrier protein is located on chromosome 2 and contains 3 exons separated by 2 intronic sequences [40]. The nuclear encoded full length PfACP has 137 residues with a molecular mass of 15.809 kDa, inclusive of a bipartite leader sequence [36,37]. In apicoplast the mature PfACP consists of 79 residues (9.00 kDa). We have chosen His-tagged protein for expression because unlike the proteins expressed in fusion with maltose binding protein (MBP) [23], cleavage of the tag, as demonstrated here, is not mandatory as the former is as active as native holo-PfACP, which, eliminates an additional step of purification improving the yield further. Moreover, we have been able to optimize the production of holo-PfACP to nearly 100% purity and in high yield in minimal medium.

Over-expression of PfACP

For the present study we have expressed the mature PfACP (residues 59–137), lacking both signal and transit sequence using PfACP-pET-28a(+) construct (Fig. 1A). The protein contains 17 additional residues at the N-terminus including hexa-His tag, which is encoded by the pET-28a(+) vector (Novagen). It is known that heterologous expression of ACP in *E. coli* gives rise to a mixture of apo-, holo-, and acyl-ACPs [18–21], and in some cases it confers toxicity to the host [18]. Co-transformation of holo-ACP synthase has been shown to improve the formation of holo-ACP [19]. Initially we attempted to express PfACP according to Waters and co-workers [22,23] but both apo- and holo-PfACPs were obtained in low yields (Fig. 1D) and had to be separated by several chromatographic steps. For the structural elucidation by NMR it is a prerequisite to have ¹⁵N

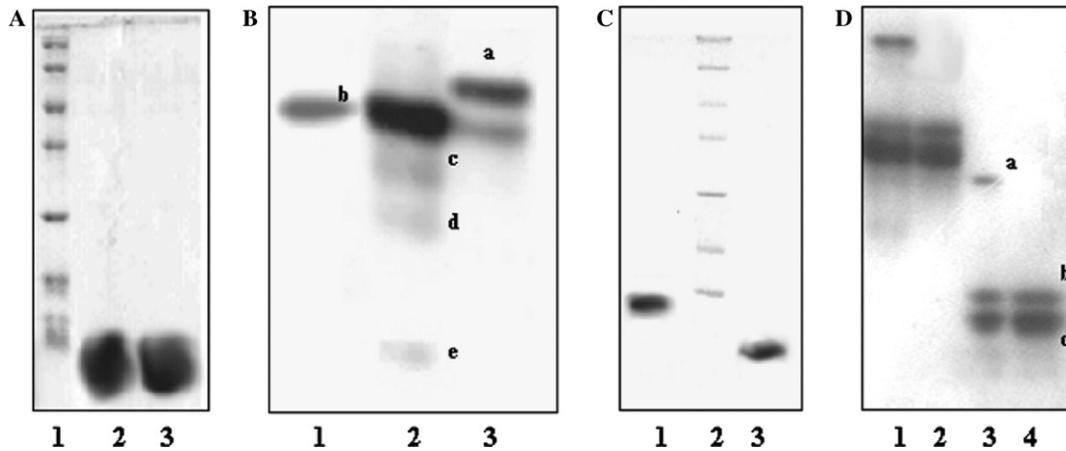


Fig. 1. (A) PfACP expression. SDS-PAGE (15%) showing the elution profile of PfACP with N-terminal His tag. Lane 1, protein marker; the protein bands correspond to 116.6, 65, 45, 35, 25, 18.4, and 14.4 kDa (from top to bottom), lanes 2 and 3, holo-PfACP eluted at 50 and 100 mM imidazole, respectively. (B) Stage specific expression of different species of PfACP. The 15% conformation sensitive gel shows the interesting pattern of appearance of different PfACP species with time after induction. Lane 1, the formation of only holo-PfACP at 9 h. Lane 2, appearance of acylated species along with that of holo-PfACP 12–14 h. Lane 3, formation of apo-form of PfACP along with holo-PfACP 14 h. Band (a) represents apo-PfACP, band (b) is holo-PfACP, and bands (c), (d), and (e) are various acyl-PfACPs. (C) Removal of His tag from recombinant PfACP. For the cleavage of His tag, 1 U of thrombin was used for 1 mg PfACP at 25 °C for 2 h. Lane 1 shows holo-PfACP with His tag, lane 2 shows protein markers, and lane 3 shows PfACP without His tag. There is a clear difference in the migration of cut and uncut holo-PfACP on 15% SDS-PAGE (2 kDa difference). (D) Native gel electrophoresis (15%) of expressed PfACP following the published protocol [23] and His tag cleaved PfACP. Removal of His tag makes the protein almost 2 kDa smaller which changes the theoretical pI value of PfACP from 5.4 to 3.9. Lane 1, His-tagged PfACP without β -mercaptoethanol; lane 2, His-tagged PfACP with β -mercaptoethanol; lane 3, PfACP without His tag with β -mercaptoethanol; and lane 4, PfACP after removal of His tag. Band (a) represents holo-PfACP dimer, band (b) is holo-PfACP, and band (c) represents apo-PfACP.

and ^{13}C labeled proteins. For this purpose we selected minimal medium to standardize the expression of pure holo-PfACP. For the labeling of PfACP, minimal medium was supplemented with ^{15}N ammonium chloride and ^{13}C glucose in appropriate amounts. Instead of growing the cells at 20 °C before induction, we chose 37 °C for initial growth until A_{600} of 1.0. Subsequent to induction the cultures were grown at 25 °C for 9–14 h. Interestingly at 9 h only holo-ACP was expressed

(Fig. 1B, lane 1). Induction for 9 h yielded 65 mg of pure holo-PfACP alone and at 12 h some acyl-ACPs were also produced (Fig. 1B, lane 2). Induction beyond 12 h showed the formation of apo-PfACP along with holo-PfACP (Fig. 1B, lane 3). Therefore, we used minimal medium for over-expression of holo-PfACP, growing at 37 °C, and shifting to 25 °C after induction for 9 h. Native PAGE with 15% polyacrylamide was found to resolve both the forms (Figs. 1B and D).

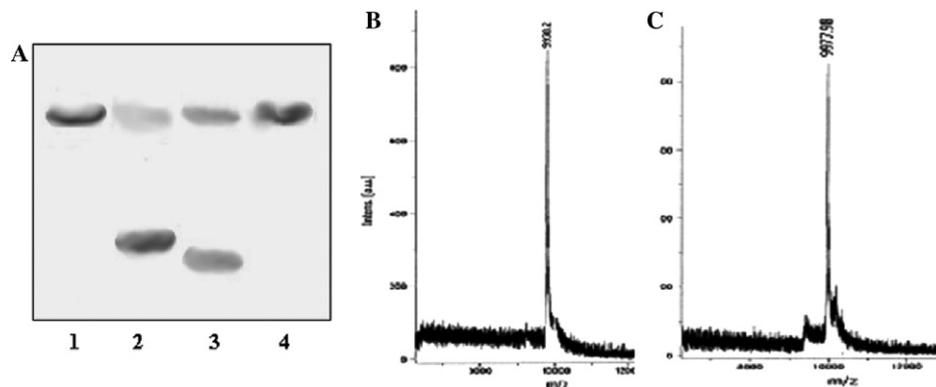


Fig. 2. (A) Acyl-PfACP synthesis (enzymatic method). *E. coli* acyl-ACP synthetase enzyme was used for the synthesis of acyl-PfACPs [28]. The extent of acylation was judged by running 15% conformational sensitive gel having 2.5 M urea. Lane 1, control lane contains only holo-PfACP without AAS enzyme; lane 2, lauryl-PfACP; and lane 3, myristoyl-PfACP. (B) MALDI spectra of C12:0-PfACP. The MALDI spectrum of the synthesized lauryl-PfACP purified on octyl-Sepharose column shows a peak at 9958.2 Da corresponding to its molecular mass. (C) MALDI spectra of C14:0-PfACP. The MALDI spectrum of the synthesized myristoyl-PfACP purified on octyl-Sepharose column shows a peak at 9977.98 Da corresponding to its molecular mass.

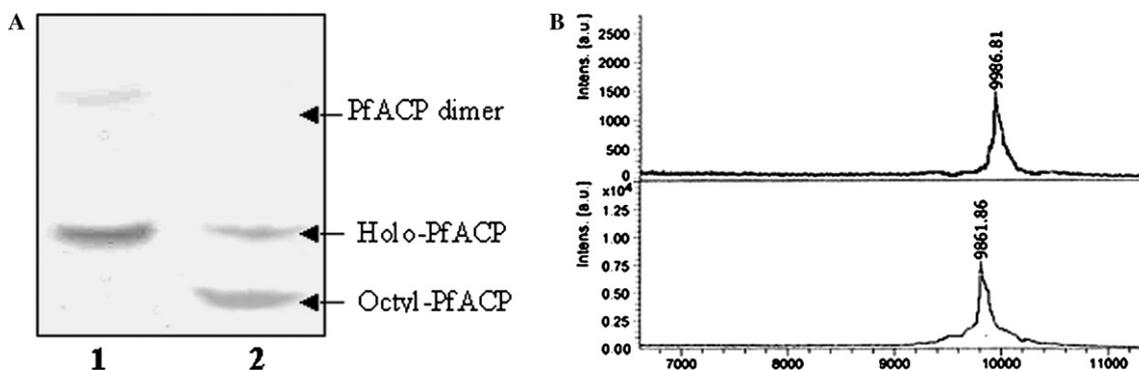


Fig. 3. (A) Synthesis of octanoyl-PfACP synthesis (chemical method). Octanoyl-PfACP was prepared using chemical method [29]. The acylated product was run on 15% native PAGE with 2.5 M urea. Lane 1 shows control holo-PfACP and lane 2 synthesized octanoyl-PfACP. (B) MALDI spectra of C8:0-PfACP, upper panel. The MALDI spectrum of octyl-PfACP synthesized using ^{15}N labeled PfACP purified on octyl-Sepharose column shows a peak at 9986.81 Da corresponding to its molecular mass. Lower panel, MALDI spectrum of ^{15}N labeled holo-PfACP with a molecular mass of 9861.86 Da.

Removal of His-tag

Holo PfACP was expressed with N-terminal His tag which is very small compared to other fusion tags like MBP, GST, etc. Therefore, the tag should not affect the structural and physical properties of the protein. To confirm this, thrombin cleavage of His-tagged PfACP was carried out, which, subsequent to a Ni-NTA affinity step, yielded homogeneous holo-PfACP (Fig. 1C). The mass of holo-PfACP with and without hexa-his tag was calculated to be 11.520 and 9.757 kDa, respectively, and the cleavage was confirmed by 15% SDS-PAGE (Fig. 1C), 15% native PAGE (Fig. 1D), ESI-MS, and MALDI-TOF TOF (data not shown). CD spectrum was taken for both tagged and the untagged ACPs. As can be seen from Fig. 4, CD spectra do not show any significant differences in their

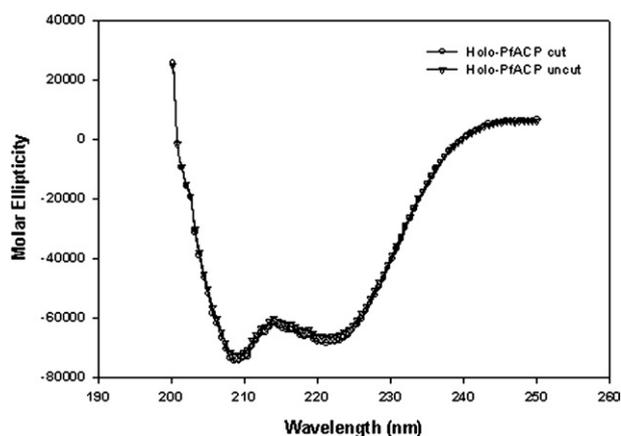


Fig. 4. CD spectrum of holo-PfACP. CD spectra of both holo-PfACP with and without His tag were recorded. The individual spectra were overlapped and extent of deviation was studied. The spectra show that both forms have very similar arrangement of secondary structural elements.

secondary structures, indicating that the his-tag does not affect the biophysical properties of the protein. Moreover, the secondary structure obtained suggests the holo-PfACP to be predominantly α -helical as is the case of ACPs from other sources [14,41]. His-tagged holo-PfACP was as active as holo-PfACP without the tag as is evident from the rate and the extent of transfer of C12 and C14 acyl groups onto it, catalyzed by *E. coli* acyl-ACP synthetase.

Preparation of acyl-PfACP

Acyl ACPs are the natural substrates for fatty acid biosynthesis enzymes but in vitro, these enzymes are able to accept acyl-CoAs and acyl-NACs with low efficiencies [38]. For a thorough characterization of *Plasmodium* FAS pathway, various acyl-PfACPs are needed. The acyl ACP synthetase gene in *P. falciparum* has not yet been annotated. We along with others [22,23] observed that *E. coli* holo-ACP synthase utilizes PfACP as a substrate, and as PfACP shares 55% identity with its *E. coli* counterpart, we speculated that *E. coli* acyl-ACP synthetase may as well accept PfACP as a substrate. Indeed, *E. coli* AAS efficiently acylated PfACP with lauric and myristic acids. The products were confirmed by conformation-sensitive gel (Fig. 2A) and also by MALDI (Figs. 2B and C). From the literature it is known that the enzymatic method works well for the synthesis of long chain acyl-ACPs [28] and chemical synthesis is suitable for short chain acyl-ACPs [29]. To check the activity of protein in chemical synthesis, we synthesized octyl ACP by chemical method and the resulting octyl-PfACP formed was confirmed by conformation sensitive PAGE and MALDI (Figs. 3A and B).

Acylation of PfACP authenticates the biochemical integrity of tagged PfACP and also provides a means for the large-scale synthesis of acyl-PfACPs to characterize plasmodial FAS enzymes.

NMR studies

For NMR studies, single (^{15}N) and double labeled (^{15}N and ^{13}C) holo-PfACP were prepared as described in the methods. The protein was uniformly labeled in both ^{15}N and $^{15}\text{N}/^{13}\text{C}$ enriched samples as indicated by three-dimensional HNCACB spectrum and MALDI

(Figs. 5C–E). The one-dimensional proton NMR spectrum acquired with doubly enriched sample is shown in Fig. 5A. The well-dispersed resonances, spanning the chemical shift range from 0.30 to 10.35 ppm in ^1H NMR one-dimensional spectrum (Fig. 5A), clearly indicate that holo-PfACP is folded. The methylene carbons of β -mercaptoethylamine, β -alanine, and hydroxymeth-

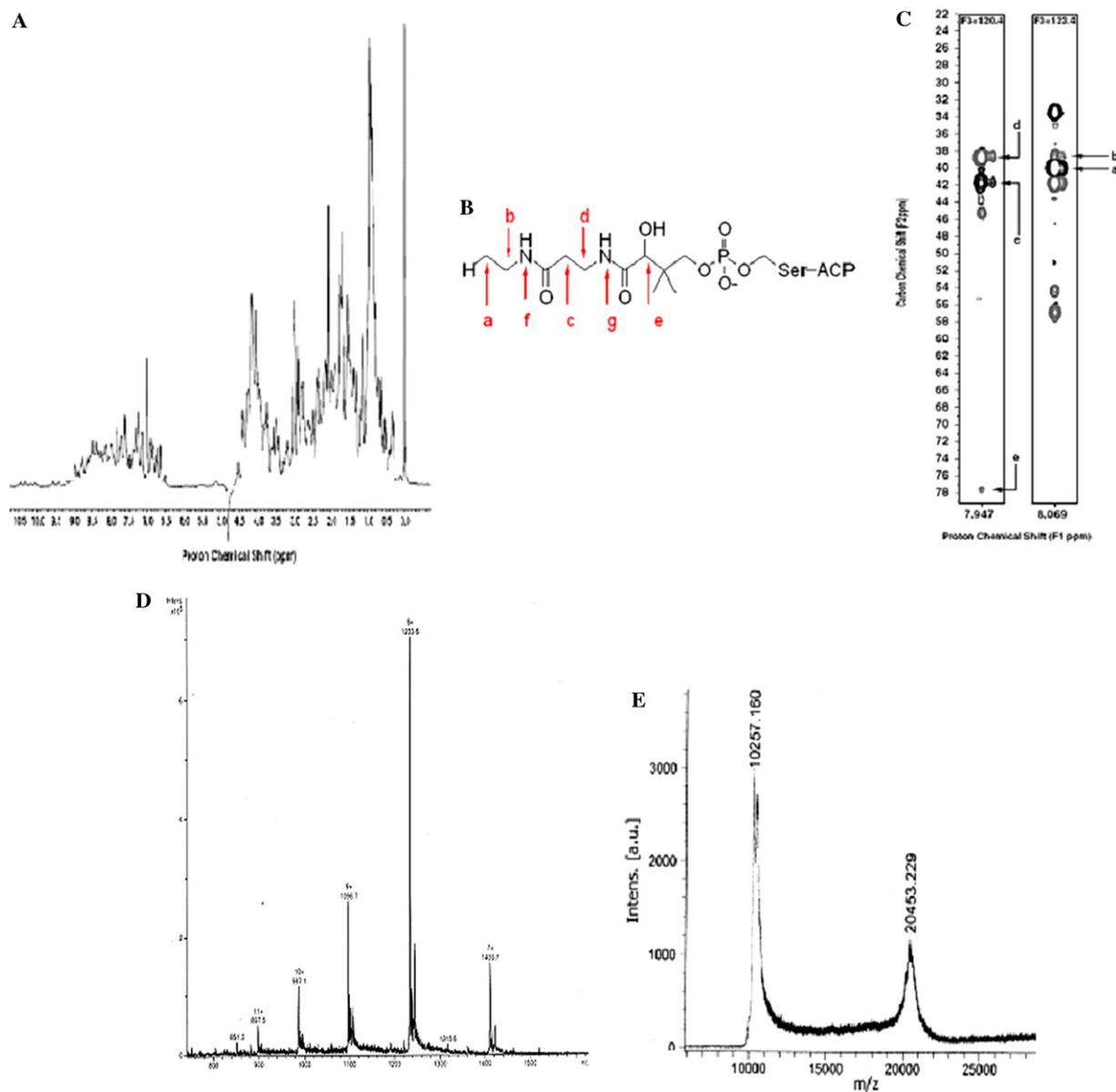


Fig. 5. (A) The one-dimensional NMR spectrum of a uniformly ^{13}C , ^{15}N enriched sample of holo-PfACP. The presence of proton signals above 8.6 ppm and below 0.6 ppm is a very clear evidence of the protein being present in a compact folded state. (B) Structure of 4-Phosphopantetheine moiety attached to ACP. (C) Three-dimensional NMR spectrum of holo-PfACP. The figure shows strip plots from the three-dimensional HNCACB spectrum. Carbon resonances corresponding to nuclei a, b, c, d, and e of the phosphopantetheine moiety are labeled in the (B). Strips are plotted at the chemical shifts (F_2) of nitrogens labeled f (123.4 ppm) and g (120.4 ppm) in the phosphopantetheine moiety (C). The assignments have been verified by co-examination of the data in the CBCA[CO]NH spectrum. (D) ESI-MS spectrum of ^{15}N labeled holo-PfACP. The mass of ^{15}N labeled holo-PfACP as observed from the ESI-MS spectrum was 9861.86 Da which is very close to the expected mass of 9865.6 Da, thus confirming that the protein was uniformly enriched in nitrogen-15. (E) MALDI spectrum of $^{15}\text{N}/^{13}\text{C}$ double labeled holo-PfACP. The mass of $^{15}\text{N}/^{13}\text{C}$ double labeled holo-PfACP as observed from MALDI spectrum was 10257.16 Da, which is very close to the expected mass of 10287.6 Da. The dimeric form of holo-PfACP was also present in the sample as can be observed from the spectrum.

ylene groups in phosphopantetheine moiety (Fig. 5B) of holo-PfACP have been assigned using the HNCACB spectrum (Fig. 5C). The unambiguous assignments of various nuclei in phosphopantetheine moiety corroborate the mass spectral data.

Conclusion

A novel method for the over-expression of PfACP in its holo form using minimal medium is reported, thus enabling the preparation of isotopically enriched protein for solution structural studies. In addition, we have also demonstrated that *E. coli* acyl ACP synthetase as well as chemical method can be used for the synthesis of acyl-PfACPs using the expressed holo-PfACP. This is important for further studies vis-à-vis characterization of PfFAS enzymes.

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