Differential Stabilities of Mefloquine-Bound Human and *Plasmodium falciparum* Acyl-CoA-Binding Proteins

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**ABSTRACT:** Toxic effects of pharmacological drugs restrict their robust application against human diseases. Although used as a drug in the combinational therapy to treat malaria, the use of mefloquine is not highly recommended because of its adverse effects in humans. Mefloquine inhibits the binding of acyl-CoAs to acyl-CoA-binding proteins of *Plasmodium falciparum* (PfACBPs) and human (hACBP). In this study, we have used molecular dynamics simulation and other computational approaches to investigate the differences of stabilities of mefloquine–PfACBP749 and mefloquine–hACBP complexes. The stability of mefloquine in the binding cavity of PfACBP749 is less than its stability in the binding pocket of hACBP. Although the essential tyrosine residues (tyrosine-30 and tyrosine-33 of PfACBP749 and tyrosine-29 and tyrosine-32 of hACBP) mediate the initial binding of mefloquine to the proteins by π-stacking interactions, additional temporally longer interactions between mefloquine and aspartate-22 and methionine-25 of hACBP result in stronger binding of mefloquine to hACBP. The higher fluctuation of mefloquine-binding residues of PfACBP749 contributes to the instability of mefloquine in the binding cavity of the protein. On the contrary, in the mefloquine-bound state, the stability of hACBP protein is less than the stability of PfACBP749. The helix-to-coil transition of the N-terminal hydrophobic region of hACBP has a destabilizing effect upon the protein’s structure. This causes the induction of aggregation properties in the hACBP in the mefloquine-bound state. Taken together, we describe the mechanistic features that affect the differential dynamic stabilities of mefloquine-bound PfACBP749 and hACBP proteins.

**INTRODUCTION**

The vast occurrence of parasite-borne malaria disease in the developing regions of the world necessitates the application of different antimalarial drugs to the patients.\(^1\) Combinatorial therapy consisting of a cocktail of drugs is used to restrict the growth and proliferation of *Plasmodium* species in the infected patients.\(^2\) Quinoline-based drugs, like chloroquine (CQ), mefloquine (MQ), and so forth, are considered as mainstream antimalarial chemotherapeutics. Quinoline drugs exert their effects by targeting different cellular machineries of *Plasmodium*.\(^3\) For example, chloroquine prevents the heme-dependent protein synthesis\(^4\) and mefloquine targets the 80S ribosome\(^5\) and dolichol kinase\(^6\) of *Plasmodium*. Among several of the quinoline-based antimalarial drugs, mefloquine is a choice because of its longer retention capacity in human body. However, mefloquine is also reported to induce cytotoxicity in the brain\(^7\) and lung\(^8\) tissues. The recent studies from our group have demonstrated the mechanisms of mefloquine-mediated inhibition of lipid binding to acyl-CoA-binding proteins of *Plasmodium* (PfACBPs) and human (hACBP). While mefloquine is a competitive inhibitor of long- and very long-chain acyl–CoAs binding to *Plasmodium falciparum* acyl-CoA-binding proteins (PfACBP16, PfACBP99, and PfACBP749),\(^9\) it also essentially blocks the binding of varying chain-length acyl-CoAs to hACBP.\(^10\) Moreover, we reported the self-aggregation of the mefloquine-bound hACBP molecules in human neuroblastoma cells.\(^10\) Because of mefloquine’s ability to bind and inactivate both PfACBPs and hACBP, it is imperative to understand the temporal dynamics of such binding events in order to analyze whether the toxicity of mefloquine overwhelms its antimalarial properties. Thus, studies are required to understand the qualitative mechanism of how mefloquine binding to PfACBPs and hACBP induces structural perturbations in the proteins that are finally transduced to generate toxicity effects in both *Plasmodium* and human cells.

The metabolic cycle of acyl-CoAs start with their synthesis from fatty acids by the catalytic activity of acyl-CoA synthetase (ACS), followed by their utilization in different forms such as energy production by beta-oxidation, synthesis of phospholipids, and energy storage as triglycerides in adipose tissue, among others.
pids and triglycerides, acylation of proteins, and so forth.11 The acyl-CoA-binding proteins (ACBPs) function in an intermediate stage of the metabolism of acyl-CoAs. ACBPs participate in the partitioning of acyl-CoAs through storage and transport of acyl-CoAs to different cellular organelles. The four-helical bundle ACBPs have conserved cavities that bind to varying chain-length acyl-CoAs.13 The ligand-binding cavities of ACBPs are mostly formed by the specific arrangement of the helix-1, helix-2, and helix-4. The molecular surface formed by 13–34 amino acid residues is primarily involved in creating the opening of cavity for the acyl-CoAs. The critical tyrosine residues in the cavities of ACBPs mediate the base-stacking interactions with the aromatic adenine moiety of coenzyme-A, thereby facilitating the binding of acyl-CoAs to ACBPs.14 The physiological roles of ACBPs include but not limited to membranogenesis,15 lipid metabolism,12 autophagy,16 and so forth. Direct binding to lipids and modulating the nuclear transcription of lipid metabolizing enzymes are the primary mechanisms of ACBPs-mediated regulation of cellular lipid homeostasis. ACBPs play a global role in the developmental processes of organisms. For example, ACBPs regulate the aging of Caenorhabditis elegans.17 Enhance water absorption by renal epithelial cells,18 and so forth. Tissue-specific isoforms of human ACBP also play a pivotal role in brain cell development19 and skin cell differentiation.20

The use of mefloquine as an antimalarial compound is based on its anti-Plasmodium functions of damaging vacuoles,21 negatively steering the protein synthesis,22 inhibiting different parasitic proteins,65 and so forth. The growth inhibitory effects of mefloquine against Plasmodium are very strong as none of the Plasmodium species are known to be resistant to this drug. Not only against malaria but mefloquine is also used as a potential drug against human cancers,23 tuberculosis,24 and so forth. Mefloquine accelerates the apoptotic death of human cells by prompting the redox stress,25 disrupting the calcium homeostasis,26 and perturbing the signaling pathways.27,28 Binding of mefloquine to connexin proteins29 and voltage-gated ion channels30 is responsible for disbalancing the cell-to-cell adhesion and membrane polarity, respectively. The toxic features of mefloquine on humans are manifested by abnormal neuropsychiatric behavior,31 cardiac muscle contraction,32 and so forth.

In this study, we have used computational methods to understand the dynamic stability of mefloquine—PfACBP749 and mefloquine—hACBP complexes. The results suggest that the temporal stability of mefloquine in the acyl-CoA-binding cavity of hACBP is higher than that of its stability in the acyl-CoA-binding region of PfACBP749. While the critical tyrosine residues of both proteins help in the initial binding of mefloquine to the proteins, the base-stacking interaction of the quinoline ring of mefloquine and tyrosine residues of hACBP is stronger and temporally longer. The mefloquine-binding residues of PfACBP749 show higher fluctuation, resulting in destabilizing movement of mefloquine in the binding pocket of the protein. On the other hand because of the less fluctuation of mefloquine-binding residues of hACBP, time-dependent repositioning of mefloquine in the binding cleft of hACBP occurs in such a way that it can participate in more favorable interactions with the neighboring residues. However, hACBP is less stable because of the transition of its N-terminal helical region to the disordered structure. The residues of the newly formed N-terminal disordered region of hACBP show higher fluctuation, and they are also involved in unfavorable steric clashes. The disordered N-terminus of hACBP also exposes previously buried hydrophobic residues to the aqueous environment, thereby transforming the protein into an aggregation-prone structure.

RESULTS AND DISCUSSIONS

Mefloquine Shows Differential Stability in the Acyl-CoA-Binding Cavities of PfACBP749 and hACBP.

Mefloquine is a small molecule modulator of hACBP and PfACBP. In the previous studies, we have described the mechanism of mefloquine-mediated competitive inhibition of acyl-CoA binding to hACBP and PfACBP749.9,10 Mefloquine binding to hACBP and PfACBP has adverse effects in human neuroblastoma cells and P. falciparum, respectively, and it is relevant to understand the aspects of spatiotemporal stability of the mefloquine—hACBP and mefloquine—PfACBP749 complexes. We conducted molecular dynamics simulation (MDS) studies of these complexes in order to find how mefloquine binding results in the differential stabilization of the proteins/complexes.

Mefloquine bound to the acyl-CoA-binding pocket of PfACBP749 (Figure 1A). The conserved tyrosine-30 (Y30) and tyrosine-33 (Y33) residues of the acyl-CoA-binding region of PfACBP749 were necessary for mefloquine binding to the protein (Figure 1A,B). The π-stacking interaction stabilized the mefloquine in the acyl-CoA-binding cavity of PfACBP749. The aromatic ring stacking interaction between mefloquine and PfACBP749 involved either of the benzene rings of Y30 or Y33 of PfACBP749 and the quinoline ring of mefloquine. While the benzene ring of the Y30 of PfACBP749 mediated the primary base-stacking interaction with mefloquine for temporally longer period, the benzene ring of Y33 also displayed transient base-stacking interaction with the quinoline ring of mefloquine. However, the simultaneous interaction of Y30 and Y33 with mefloquine was not observed. Two additional residues of the acyl-CoA-binding region, arginine-23 (N23) and leucine-27 (L27), showed hydrophobic interactions with the piperidine ring of mefloquine. The lysine-26 (K26) participated in a hydrogen bond with the hydroxyl group of mefloquine. In general, the preliminary binding of mefloquine to PfACBP749 was stabilized by these combination of aromatic, hydrophobic, and polar interactions. However, the stability of mefloquine in the binding pocket of PfACBP749 gradually decreased with the progression of MDS. This phenomenon was associated with the events of breaking and making of bonds. The hydrophobic interactions of N23 and L27 with the piperidine ring of mefloquine were temporally substituted by the hydrophobic interactions of arginine-13 (N13) and valine-9 (V9) (Figure 1A,B). In the process of reconfiguration of mefloquine conformation in the ligand-binding region of PfACBP749, the aromatic base-stacking interaction between mefloquine and Y30 was lost (Figure 1A,B). Loss of this interaction was compensated by multiple hydrophobic interactions between the isoleucine-12 (I12), leucine-15 (L15), and proline-16 (P16) of PfACBP749 and the quinoline ring of mefloquine. The hydroxyl group of tyrosine-75 (Y75) formed a hydrogen bond with the hydroxyl group of mefloquine in the later stages of MDS (Figure 1A,B). These later interactions were long-lasting, and they completely outcompeted the previous set of interactions between mefloquine and PfACBP749 (Figure 1C). While the initial binding of mefloquine to PfACBP749 involved its interactions with the residues of the helix-II of the protein, the new interactions of mefloquine in the binding
region allowed it to interact with the residues of the helix-I of the protein. The outcome was the repositioning of mefloquine in a new site of the binding cavity (Figure 1C). In fact, mefloquine binding to the new region of PfACBP was more stable than the previous position because of a greater number of interactions of mefloquine with the residues of PfACBP (Figure 1D).

Like its binding to the PfACBP, mefloquine occupied a position in the acyl-CoA-binding region of hACBP. The benzene rings of tyrosine-29 (Y29) and tyrosine-32 (Y32) of hACBP had simultaneously and equally contributed to the base-stacking and hydrophobic interactions with the quinoline and piperidine rings of mefloquine (Figure 2A,B). The methionine-25 (M25) and arginine-14 (R14) showed hydrophobic and polar interactions with the aromatic and fluoride groups of the mefloquine, respectively (Figure 2A,B). Because of the cumulative effects of the aromatic base-stacking interactions of Y29 and Y32 with mefloquine, the initial binding of mefloquine to hACBP was more stable than the mefloquine binding to PfACBP. During the progression of MDS, the stability of mefloquine in the binding region of hACBP had increased even more than its initial binding to the protein. Unlike the mefloquine in PfACBP, the mefloquine in the ligand-binding cleft of hACBP did not change its position. Instead, it reoriented its piperidine and quinoline rings in such a way so that they could involve themselves in more favorable interactions. This was manifested by the base-stacking interaction of Y29 with the quinoline ring, hydrophobic interactions by M25 and proline-20 (P20), and two polar contacts by the aspartate-22 (D22) with the nitrogen of the piperidine ring and the hydroxyl group of mefloquine (Figure 2A,B). It was noteworthy that polar connections of D22 existed longer than the aromatic interactions of Y29 (Figure 2C). The strong interaction of D22 with mefloquine was responsible for maintaining the localization of mefloquine in its initial binding position throughout the MDS time (Figure 2C). This event had compelled mefloquine to continuously interact with the residues of the helix-II of the protein. Mefloquine displayed relatively constant number of bonds with the hACBP protein (Figure 2D), implying that no robust bond breaking and making occurred during the simulation time.

The differential stability of mefloquine in the binding pockets of PfACBP and hACBP was based on the mechanisms of bond-rotational/torsional and translational motion-mediated repositioning of mefloquine. Because of complex spatiotemporal rearrangement of interactions between mefloquine and the residues of PfACBP, a bond torsion

Figure 1. Time-dependent mefloquine repositioning in the binding cavity of PfACBP occurs because of its differential binding with different residues of proteins during the later stages of molecular dynamics simulation. (A) 0 and 190 ns structures of mefloquine-bound PfACBP show that mefloquine repositions itself in a different region of binding cavity in the final stages of MDS than its initial binding site of the proteins. (B) Interaction diagrams of mefloquine and residues of PfACBP in the 0 and 190 ns structures of mefloquine–PfACBP complexes. (C) Interaction fraction of contacts of mefloquine and PfACBP residues. The stacked bar charts are normalized over the 200 ns of the trajectory. (green: H-bonds, violet: hydrophobic interactions, purple: magenta: ionic interaction, and blue: water bridge). 0 and 190 ns positions of mefloquine in the binding cavity of PfACBP. (D) Total number of contacts between mefloquine and PfACBP throughout the simulation time.
followed by a rotational movement of the molecule along an imaginary two-fold rotational axis (i2) event was observed in PfACBP749-bound melloquine. The bond torsion occurred because of the rotation of the C11−C12 bond of melloquine (Figure 3A). Because this bond was connecting the piperidine and quinoline rings of melloquine, the high degree of freedom of rotation of this bond imparted greater flexibility to the two heterocyclic groups. However, the translational fluctuation [represented by root mean square fluctuation (RMSF)] of

Figure 2. Melloquine does not drastically change its position in the binding cavity of hACBP during the simulation time because of continuously interacting with specific residues of the protein. (A) 0 and 190 ns structures of melloquine−hACBP complex show that the melloquine molecule is not considerably shifted from its initial binding site of the cavity throughout the simulation time. (B) Molecular contact diagrams of melloquine with the residues of hACBP in the 0 and 190 ns structures of melloquine−hACBP complex. (C) Interaction fraction of contacts of melloquine and hACBP residues. The stacked bar charts are normalized over the 200 ns of the trajectory. (green: H-bonds, violet: hydrophobic interactions, purple: magenta: ionic interaction, and blue: water bridge). 0 and 190 ns positions of melloquine at the binding cavity of hACBP. (D) Total number of intermolecular contacts between melloquine and hACBP throughout the simulation time.

Figure 3. Melloquine binding is unstable in the binding cavity of PfACBP749 compared to its binding to the cavity of hACBP. (A) (Left) Atom numbers of the quinoline and piperidine rings of melloquine molecule. (Right) Quantification of the torsion angles of C11−C12 bonds of PfACBP749- and hACBP-bound melloquine during the simulation time. (B) (Upper left) RMSF values of the atoms of PfACBP-bound melloquine. (Upper right) RMSD values of PfACBP-bound melloquine. (Lower) Representation of bond torsion and rotation of melloquine that results in its movement from the initial position to final position in the cavity of PfACBP749. (C) (Upper left) RMSF values of hACBP-bound melloquine. (Upper right) RMSD values of hACBP-bound melloquine. (Lower) Representation of bond torsion and rotation of melloquine that results in its very less fluctuation from the initial position to final position in the binding cavity of hACBP.
atoms (C11 and C12) participating in this bond was lowest (C11_RMSF = 1.905 Å and C12_RMSF = 1.704 Å) (Figure 3B). The overall RMSF of the atoms of mefloquine was in the range of 1.704−5.362 Å. The RMSF of quinoline ring atoms (N1, C4, C5, and C6) was highest (N1_RMSF = 4.278 Å, C4_RMSF = 4.785 Å, C5_RMSF = 5.362 Å, and C6_RMSF = 5.194 Å) (Figure 3B) because of the drastic shift of the quinoline ring from its initial position. Owing to the significant displacement, the mefloquine molecule was not very stable in the binding cavity of the PfACBP749. The root mean square deviation (RMSD) values of mefloquine in the mefloquine–PfACBP749 complex were in the limits of 0.639−11.018 Å (Figure 3B). The attainment and maintenance of high RMSD values (>6 Å) in the later phase of MDS also showed the destabilization of mefloquine in the acyl-CoA-binding pocket of PfACBP749.

The movement of mefloquine in the binding cavity of hACBP also comprised two discrete steps during the progress of MDS. The first step was the torsion of the C11−C12 bond, followed by a rotation of mefloquine along an imaginary axis (Figure 3A,C). However, the later rotational movement of mefloquine was stabilizing rather than destabilizing. It positioned the piperidine and quinoline rings of mefloquine in such a position that it could pursue more stabilizing interactions with P20 and D22, other than sustaining the indispensable interactions with M25 and Y29. The lower fluctuation of mefloquine in the binding region of hACBP was observed by lower RMSF values of its atoms which were in the range of 2.113−4.254 Å. The three lowest fluctuations were observed for C10, C11, and C12 atoms (C10_RMSF = 2.113 Å, C11_RMSF = 2.282 Å, and C12_RMSF = 2.352 Å), whereas quinoline’s C7 atom showed the highest RMSF value (C7_RMSF = 4.254 Å) (Figure 3C). The relative stability of mefloquine in the mefloquine–hACBP complex was prominent by mefloquine’s lower RMSD values (0.16−5.57 Å) (Figure 3C). Together, these results described the mechanism of variable spatiotemporal stability of mefloquine in its PfACBP749- and hACBP-bound forms.

**hACBP is less Stable than the PfACBP749.** In the next phase of the study, we investigated the stabilities of the PfACBP749 and hACBP proteins in their mefloquine-bound states. Though mefloquine was unstable in the binding pocket of PfACBP749 than in the binding cleft of hACBP, the overall stability of PfACBP749 protein was observed to be higher than the stability of hACBP protein (Figure 4A). The higher stability of PfACBP749 backbone was manifested by lower RMSD values which equilibrated to maximum at the later phase of MDS (Figure 4B). The RMSD values of the backbone of PfACBP749 were in the range of 1.141−4.63 Å. On the other hand, the backbone of hACBP had undergone frequent destabilization which was revealed by its higher RMSD values (Figure 4B). The RMSD values of the backbone of hACBP were in the range of 0.904−5.406 Å. hACBP was observed to lose secondary structures of its N-terminal region. The N-terminal residues of hACBP, which were in the first helix of the protein during the initial period of MDS, gradually lost their secondary structure to become part of a newly formed disordered region (henceforth called loop-N) (Figure 4A). In both the proteins, the unstable regions were the disordered loops that connected the helix-II and helix-III of the proteins (henceforth called loop-II) (Figure 4A,B). These loops represented the 38th−52nd residues of PfACBP749 and 37th−49th residues of hACBP, and the RMSF values of these loops were in the range of 1.37−4.34 and 2.22−5.03 Å, respectively (Figure 4C). Other than this loop, the N-terminal residues of hACBP were significantly unstable. The RMSF values of the residues (serine-2 to alanine-10) of a newly formed N-terminal disordered region of hACBP were in the range of 2.34−6.7 Å (Figure 4C). Higher fluctuation of these residues was due to the disordered nature of the region. Unlike PfACBP749, the acyl-CoA-binding residues of hACBP did not show high fluctuation.
The PfACBP749 structure was robust in terms of retaining its secondary structures throughout the simulation time (Figure 5A). The span of individual helices of PfACBP749 were observed to be intact during the MDS. Even though the fluctuation of the residues of PfACBP749 was comparatively lower than the residues of hACBP, fluctuation of the key mefloquine-binding residues was higher in PfACBP749. The fluctuation of loop-II has transduced its effects in locally destabilizing the residues of helix-II that were involved in binding to mefloquine. For example, the values of RMSF_{PfACBP749-Y30}/hACBP-Y29 = 2.05, RMSF_{PfACBP749-Y33}/hACBP-Y32 = 1.77, and RMSF_{PfACBP749-K34}/hACBP-K33 = 1.21 showed that the mefloquine-binding residues were more unstable in the PfACBP749. The high RMSF values of Y30, Y33, and K34 of PfACBP749 also led to higher deviation of the side chains of these residues in the final phase of MDS. The temporal increase of entropy values of the side chains of the mefloquine-binding residues of PfACBP749 during MDS correlated with the higher RMSF values of these residues (Figure 5B). The higher RMSF coupled entropy values of the residues of helix-II resulted in increased steric clashes of these residues. Other than the steric clashes of mefloquine-binding residues, lysine-31 (K31) ↔ valine-9 (V9), tyrosine-32 (Y32) ↔ alanine-59 (A59), and phenylalanine-5 (F5) showed steric clashes with arginine-74 (R74) (Figure 5C).

The modulation of hACBP structure was more prominent than PfACBP749. The helical-to-disorder transition of the N-terminal region of hACBP occurred in the later stages of MDS (Figure 6A). The induction of disordered structure was

![Figure 5. Mefloquine-binding residues of PfACBP749 are unstable without changing the secondary structure. (A) Proportion of secondary structure components of PfACBP749 during the simulation time. (B) Conformational entropy of the residues of 0 and 190 ns PfACBP749 structures. (Inset) The entropy of the mefloquine-binding residues are higher in the 190 ns structure in comparison to the 0 ns structure of the protein. (C) Steric clashes of the residues of 190 ns PfACBP749 structures. The mefloquine-binding residues (K31 and Y32) are involved in unfavorable steric clashes with A59 and R74 residues of the protein.](image)

![Figure 6. Instability of the N-terminal region of hACBP is coupled to its transition from helical to disordered structures. (A) Secondary structure composition of hACBP during different time-points of MDS. (B) Conformational entropy of the residues of hACBP at 0 and 190 ns time-points of simulation. (Inset) The conformational entropy of the residues of 190 ns hACBP structure is higher than the conformational entropy of these residues during the initial period of simulation. (C) Steric clashes of the residues of 190 ns structure of hACBP. The N-terminal residues (S2 and A4) participate in steric clashes with E79 and I75 of the protein, respectively.](image)
and formation of puncta-like aggregates of hACBP in human neuroblastoma cells. In order to understand the intricate mechanisms underlying that phenotype, we took advantage of this simulation study. While there are various mechanisms in which proteins aggregate, one plausible mechanism is the intermolecular interactions due to collapse of solvent-exposed hydrophobic patches of proteins. We explored whether this was the mechanism of aggregation of mefloquine-bound hACBP. Analysis of the sequences revealed that hACBP, but not PfACBP749, had a hydrophobic patch in its N-terminal region (Figure 7A). Because the N-terminus of hACBP could undergo transition to disordered structure, leading to its destabilization, it was reasonable to understand that the N-terminal hydrophobic patch of hACBP was responsible for the protein’s self-association process. An analysis of the solvent-accessible surface area (SASA) of hACBP also revealed that the N-terminal region of mefloquine-bound unstable hACBP structure had higher SASA than the SASA of N-terminal region of mefloquine-bound hACBP structure of early phase of simulation (Figure 7B). This meant that the structural unfolding of the N-terminal region of mefloquine-bound hACBP gradually opened this region in a protein breathing-like phenomenon, leading to expose the N-terminus to water only in its unstructured form. It was evident that the disordered hydrophobic patch of the N-terminus of hACBP was more exposed to the aqueous environment in the mefloquine-bound state. This event had decreased the stability of hACBP even further as evident from the gradual loss of free energy of the protein during MDS (Figure 7C). The cumulative result of exposure of the N-terminal hydrophobic region to water and overall lower stability of the protein are the causes of generation of aggregation properties in the hACBP protein. The N-terminus of unstable hACBP showed higher aggregation potential (Figure 7D) (the aggregation index measured the potential of aggregation of every residues in the proteins). So, a series of events consisting of destabilization of hACBP structure, transition of a segment of helix-I to disorder, and exposure of N-terminal hydrophobic segment to the aqueous environment resulted in the aggregation of mefloquine-bound hACBP.

A hydrophobic region (residue 33–38) was also observed in the acyl-CoA/mefloquine-binding region of PfACBP749 (Figure 7A). During the progress of simulation, this region was also observed to be capable of being exposed to water (Figure 7B), resulting in temporal decrease of free energy of the protein (Figure 7C). As a result of this, the mefloquine-binding region was theoretically converted to the aggregation-prone region (Figure 7D). However, in our previous studies with mefloquine binding to PfACBPs of P. falciparum, we did not observe aggregation of mefloquine-bound PfACBPs (including mefloquine–PfACBP749 complexes). The reason of this apparent contradiction of results could be imparted to the binding of acyl-CoAs or mefloquine to the aggregation-prone cavity of PfACBP749. Binding of acyl-CoAs or mefloquine to this hydrophobic region could shield this region from being exposed directly to the water, thereby preventing the aggregation of the protein.

### CONCLUSIONS

Interaction of drugs with Plasmodium and human proteins defines their usage in antimalarial therapeutics. While the most extensively used quinoline-class of antimalarial drugs, such as chloroquine, mefloquine, amodiaquine, and so forth, target different Plasmodium proteins and machineries to prevent the growth and multiplication of the organism, they also cause toxicity to human cells by inactivating proteins or perturbing signaling cascades. Even though the high bioavailability of mefloquine makes it a good choice for treating the acute cases of malaria prophylaxis, its prolonged use causes adverse effects in humans. In light of our recent findings that mefloquine binds and inactivates both PfACBP and hACBP, it is crucial to understand the comparative dynamics of mefloquine binding to these proteins in order to analyze whether the toxicity effects of mefloquine overrides its activity as a drug. This study is aimed to explore that aspect, and we describe that mefloquine binding to PfACBP749 is less stable than its binding to hACBP, whereas the stability of hACBP is lower.

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**Figure 7.** Destabilization of the N-terminal region of mefloquine-bound hACBP is responsible for inducing aggregation-prone properties in the protein. (A) Sequence-based hydrophobicity of different segments of PfACBP749 and hACBP proteins. (Black arrow) The N-terminal region of hACBP is highly hydrophobic. (B) Difference of SASA of 190 and 0 ns structures of PfACBP749 and hACBP. (Inset) The high SASA of the N-terminal region of 190 ns structure of hACBP. (C) Free energy of different time-point structures of PfACBP749 and hACBP. (D) Aggregation index of 190 ns structures of PfACBP749 and hACBP. (Black arrow) The aggregation index of the N-terminal residues of 190 ns hACBP structure is very high. (Lower) Aggregation-prone regions of PfACBP749 and hACBP.
than that of PfACBP749. The melofloquine-bound hACBP structure is also vulnerable to self-aggregation. The reason that melofloquine is retained in human body for longer period can be explained considering its binding to hACBP. Melofloquine in the acyl-CoA-binding cavity of hACBP is continuously stabilized by tyrosine-mediated base-stacking interactions and several other hydrophobic and polar interactions. Less fluctuation of the residues of helix-II of hACBP allows a temporally stronger and longer binding of melofloquine to the hACBP cavity. On the contrary, the binding of melofloquine to PfACBP749 is comparatively less stable than its binding to hACBP. The higher fluctuation of the residues of helix-II of PfACBP749 disrupts the essential base-stacking interaction of melofloquine with the tyrosine residues of the protein. A time-dependent induction of a new set of hydrophobic interactions between the melofloquine and residues of helix-I reposition melofloquine in a new location of the protein’s cavity. Though these newly formed hydrophobic interactions are still able to hold melofloquine in the acyl-CoA-binding cavity of PfACBP749, the affinity of melofloquine to PfACBP749 gradually decreases with time because of higher fluctuation of the melofloquine molecule in the cavity.

Although melofloquine binding is stronger to hACBP, the melofloquine-bound hACBP attains lower stability at different time-points in comparison to melofloquine-bound PfACBP749. The reason of such instability of hACBP is the helical-to-disorder structural transition of the N-terminus during the protein’s binding to melofloquine. The N-terminal region of hACBP contains a hydrophobic patch, and the loss of its helical structure exposes this patch to aqueous environment. This phenomenon results in increasing the aggregation-prone properties of the melofloquine-bound hACBP.

One interesting question is how the N-terminal region of melofloquine-bound hACBP unfolds from its helical structure. We understand that the unfolding of helix-I is conducive because of its positioning at the N-terminal region (less structural constrains on the edge of the protein) and clustering of hydrophobic amino acids in this region. The ends of proteins are usually more dynamic in nature, and they show structural flexibility in terms of converting one kind of secondary structure to another depending upon the changing conditions. So, we hypothesize that melofloquine-bound hACBP could have undergone such kind of structural transition because of various reasons such as steric clashes, exposure of hydrophobic regions to water, transient breathing of helical structure, and so on.

Melofloquine-bound PfACBP749 is not aggregation-prone. Two possible mechanisms prevent the transformation of PfACBP749 into an aggregation-prone structure. First, the N-terminus of PfACBP749 does not contain any hydrophobic segment. Second, the residues of the N-terminal helical region interact with the melofloquine in the later phase of MDS. Both of these can resist the structural alteration of the N-terminal region of PfACBP749. Moreover, due to the instability of melofloquine in the binding cavity of PfACBP749, there could be a dissociation of melofloquine from the melofloquine–PfACBP749 complex, leading to the generation of apo-protein and subsequent acyl-CoA binding to PfACBP749. The apo-PfACBP749 and the acyl-CoA–PfACBP749 complex can be stable, and they are probably not subjected to aggregation.

The free-energy loss due to the structural instability of hACBP is compensated by the binding of melofloquine to hACBP. Hence, only the hACBP protein becomes transiently less stable in the melofloquine–hACBP complex. The possibility is that hACBP in the melofloquine–hACBP complex attains a higher local free-energy minima in the energy landscape due to the unfolding of the N-terminal helical region. In this process, the evolution of an intermediate transition (metastable) state converts the native proteins into an aggregation-prone structure. The aggregated melofloquine-bound hACBP protein is in lowest free-energy minima. There are several other examples which demonstrate that binding of ligands to proteins causes structural changes in such a way that metastability is induced in the proteins, followed by aggregation of the metastable protein complex.33

The results of this study can be impactful in understanding how small molecule modulators differentially target orthologous human and Plasmodium proteins. The leads that melofloquine occupies nonidentical positions in the cavities of hACBP and PfACBP749 require chemical modifications of melofloquine to generate adducts that are more potent against PfACBPs compared to hACBP. Investigations on the mechanism of aggregation of melofloquine-bound hACBP can also be a good prospect for future studies.

Taken together, we conclude that melofloquine’s toxic effects against hACBP subdue its application as an anti-Plasmodium drug. Because melofloquine-mediated competitive inhibitory function is more potent against hACBP than PfACBP749 and melofloquine binding transforms hACBP into an aggregation-prone structure, the application of melofloquine can be more adverse in human than its parasiticidal functions.

## METHODS

### Structures.

The ligand-free structure of hACBP was obtained from protein data bank (PDB code: 2FJ9).34 The apo-PfACBP749 structure was generated by extracting the protein coordinates from the PfACBP749–myristoyl-CoA complex (PDB code: 1HBK).14

**Molecular Docking.** The protein structures were corrected in the protein preparation wizard of Maestro 9.2 (Schrödinger Inc.) as carried out in our earlier studies.35–37 The accurate structures of the proteins were generated by adding missing hydrogen atoms, making optimal protonation states of histidine residues, and assigning proper bond orders with the following refinement parameters—OPLS_2005 forcefield, convergence heavy atom to RMSD of 0.30 Å. This was followed by minimization of the structures in the Molecular Modelling Toolkit (MMTK).38 Amber10 parameters were used in the minimization steps. Steepest descent with the conjugate gradient minimization involved $10^4$ iterations. Docking of melofloquine upon the PfACBP749 and hACBP structures was carried out in the standalone version of Molegro Virtual Docker. The melofloquine structure was refined to assign bond orders and hybridization states, create explicit hydrogen atoms, assign partial charges, and detect the flexible bond torsions between the atoms. The potential melofloquine-binding cavities on the surface of the proteins were detected by running two sequential algorithms—“create surface (generates electrostatic surface of the protein)” and “detect cavity.” The surfaces of the proteins were generated at 0.7 Å resolution with a probe radius of 1 Å. The cavity detection program identified expanded van der Waals regions on the surface of the proteins (cavity detection parameters—maximum cavity volume: $10^4$ Å$^3$, probe size: 1.2, maximum number of ray checks: 16, minimum number of ray hits: 12, grid resolution: 0.8). Rigid-body docking of melofloquine on manually assigned cavities of the...
The protein–mefloquine interactions were also analyzed in a simulation interaction diagram generator. The geometric criteria for protein–mefloquine H-bond was: distance of 2.5 Å between the donor and acceptor atoms (D—H···A); a donor angle of >120° between the donor-hydrogen-acceptor atoms (D—H···A); and an acceptor angle of >90° between the hydrogen-acceptor bonded atom (H···A—X). Hydrophobic interactions were π-cation, π–π, and other nonspecific interactions. The geometric criteria for hydrophobic interactions were as follows: π-cation—aromatic and charged groups within 4.5 Å, π–π—two aromatic groups stacked face-to-face or face-to-edge, and other—a nonspecific hydrophobic side chain within 3.6 Å of a ligand’s aromatic or aliphatic carbons. Ionic interactions or polar interactions were between two oppositely charged atoms that were within 3.7 Å of each other and did not involve a hydrogen bond.

The SASA and the time-dependent changes in free energy of the structures were measured in the simulation event analysis program of Maestro 9.2 (Schrodinger Inc.).

**Protein–Ligand Interaction.** The interaction maps of mefloquine with PfACBP749 and hACBP were generated in the LigPlot + software.

**Secondary Structure Analysis.** Analysis of the secondary structure components of the proteins was carried out in the 2Struc Web server.

**Conformational Entropy Analysis of Protein Residues.** The residue-wise conformational entropy of PfACBP749 and hACBP at different time-points of MDS was quantified in the PLOPS Web server.

**Steric Clash Analysis.** Analyses of steric clashes of the residues of PfACBP749 and hACBP structures were performed by using the MolProbity program.

**Aggregation Property Analysis of Protein Residues.** The identification of aggregation-prone regions in the PfACBP749 and hACBP structures was carried out by quantitating the aggregation index of different regions of the structures in the Aggregan3D Web server.

**Hydropathy Index Analysis.** The sequence-dependent hydrophobicity of different segments of PfACBP749 and hACBP was measured in the ProtScale ExPASy Web server (https://web.expasy.org/protscale/).

**Structure Preparation and Visualization.** Mefloquine structure was drawn and prepared in MarvinSketch (ChemAxon). Protein and protein—ligand complex structures were visualized and analyzed in PyMol.

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Notes
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