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Structural Characterization of the Regulatory Domain of Brain Carnitine Palmitoyltransferase 1

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Abstract

Neurons contain a mammalian-specific isoform of the enzyme carnitine palmitoyltransferase 1 (CPT1C) that couples malonyl-CoA to ceramide levels thereby contributing to systemic energy homeostasis and feeding behavior. In contrast to CPT1A, which controls the rate-limiting step of long-chain fatty acid β -oxidation in all tissues, the biochemical context and regulatory mechanism of CPT1C are unknown. CPT1 enzymes are comprised of an N-terminal regulatory domain and a C-terminal catalytic domain that are separated by two transmembrane helices. In CPT1A, the regulatory domain, termed N, adopts an inhibitory and non-inhibitory state, N α and N β , respectively, which differ in their association with the catalytic domain. To provide insight into the regulatory mechanism of CPT1C, we have determined the structure of its regulatory domain (residues Met1-Phe50) by NMR spectroscopy. In relation to CPT1A, the inhibitory N α state was found to be structurally homologous whereas the non-inhibitory N β state was severely destabilized, suggesting a change in overall regulation. The destabilization of N β may contribute to the low catalytic activity of CPT1C relative to CPT1A and makes its association with the catalytic domain unlikely. In analogy to the stabilization of N β by the CPT1A catalytic domain, non-inhibitory interactions of N of CPT1C with another protein may exist.

Keywords

Allosteric enzymes; energy homeostasis; fatty acid metabolism; NMR spectroscopy; membrane proteins

INTRODUCTION

The enzyme carnitine palmitoyltransferase 1 (CPT1) mediates the transport of long-chain fatty acids (LCFA) across intracellular membranes by converting long-chain acyl-CoA to acyl-carnitine.^{1,2} This reaction represents the rate-limiting step in mitochondrial fatty acid β -oxidation in all tissues. The closely related liver and muscle isoforms, CPT1A and CPT1B, are anchored to the outer mitochondrial membrane (OMM) by two transmembrane (TM) helices that separate the small N-terminal regulatory domain, termed N, from the large C-terminal catalytic domain (CD; Figure 1A).³ In CPT1A, N and CD both face the cytosol (Figure 1A).³ Malonyl-CoA (MCoA), the first intermediate in fatty acid synthesis, inhibits CPT1 activity, thereby regulating cellular fuel consumption.^{4,5} In mammals exists a third isoform, CPT1C that is exclusive to neurons.^{6,7} CPT1C has been implicated in the regulation of ceramide levels in the ER of neurons without contributing directly to their *de novo* synthesis.^{8,9} In the arcuate nucleus of the hypothalamus, CPT1C couples the MCoA

concentration to ceramide levels.⁹ The hypothalamus is a critical regulator of systemic energy homeostasis, and CPT1C knock-out mice show decreased food intake but then higher susceptibility to obesity and diabetes when fed a high fat diet.^{10,11} In hippocampal neurons, a CPT1C-mediated increase in ER ceramide level controls dendritic spine maturation and CPT1C-deficient mice exhibit deficiencies in spatial learning.⁸ Thus, in neurons where fatty acids are not a significant fuel source,¹² MCoA and CPT1C partake in important physiological regulatory events that render CPT1C a target for the pharmacological control of obesity.¹³

CPT1C shares sequence identities of 51.7 and 50.6% with CPT1A and CPT1B, respectively, which indicate homologous backbone structures. CPT1C binds MCoA with the same affinity as CPT1A but its acyltransferase activity was found to be lower by a factor of 20–300 in comparison.⁷ CPT1C localizes predominantly to the endoplasmic reticulum (ER).⁷ Based on the membrane topology of CPT1A,³ it is expected that N and CD reside on the same side of the membrane, most likely facing the cytosol (Figure 1A). Palmitoyl-CoA has been identified as a substrate for CPT1C.⁷ However, because of its low catalytic activity,^{6,7,10} it is presently unclear whether palmitoyl transfer to carnitine represents the physiological reaction catalyzed by CPT1C or this ability is a remnant of its evolutionary origin, CPT1A gene duplication.⁶ Metabolomic profiling of CPT1C knock-out versus wild type mice revealed differences in endocannabinoid metabolism, changes in the levels of carnitine, its metabolites, and oxidized glutathione, but not fatty acid metabolism.¹⁴ On the other hand, non-neuronal tumor cells that constitutively express CPT1C show increased fatty acid oxidation, ATP production, and resistance to glucose deprivation or hypoxia.¹⁵ These findings highlight the need to understand the regulation of CPT1C in relation to CPT1A to provide insight into their different apparent catalytic activities. We report here the structural characterization of the regulatory N domain of CPT1C by multidimensional, heteronuclear NMR spectroscopy and its comparison to N of CPT1A to evaluate possible differences and similarities in regulation between the two isoforms.

The regulatory domain of CPT1A can exist in two structural states, termed N α and N β , which are inhibitory and non-inhibitory, respectively.¹⁶ CPT1A not only responds to MCoA concentration, which mirrors the short-term metabolic state, but also to OMM enzyme location (membrane curvature) and OMM fluidity and composition, which relate to the long-term metabolic state. We have proposed that these factors are integrated into one regulatory signal by setting the prevalent N α :N β ratio via an inhibitory OMM·N α ·MCoA·CD complex and a catalytically active N β ·CD·LCFA-CoA·carnitine complex.¹⁶ In other words, in conjunction with MCoA concentration, the affinity of N for the CD relative to the OMM surface determines the catalytic activity of CPT1A. Despite its relatively small size of 42 residues, N β displays a quite complex structure, consisting of sheets β 1- β 2 and helix α 2 (Figure 1B). Its structural integrity depends on the availability of a distinctly curved, amphiphilic binding surface. If this environment is unavailable, the N α state emerges by default.¹⁶ Structurally, N α is characterized by the loss of the anti-parallel β -sheet and the emergence of propensity for an N-terminal helix, termed α 1 (Figure 1B). Other than the structure determination of N of CPT1A, structural studies of CPT1 enzymes have not been achieved, perhaps relating to their membrane association (Figure 1A). With the many unknowns still surrounding the biochemical and cellular context of CPT1C function, the present study uses structural data to identify and discuss the possible regulatory scenarios available to CPT1C.

RESULTS

Solution Conditions and Influence of Peptide Length on N Structural Parameter

To study the regulatory domain of CPT1C, again termed N, by NMR spectroscopy, two isotope-labeled peptides, encompassing Met1-His42 and Met1-Phe50 of CPT1C (Figure 1C), were prepared recombinantly. N_{M1-F50} stops before the first predicted TM helix (Gly53-Leu75) and N_{M1-H42} corresponds to the peptide used to study N of CPT1A.¹⁶ Based on sequence similarities and identities of 76.2 and 57.1%, respectively, for the N-terminal 42 residues of CPT1A and CPT1C (Figure 1C), we employed the conditions that stabilize $N\alpha$ and $N\beta$ of CPT1A to promote these states in N of CPT1C. For CPT1A, the amphiphilic, bent $N\beta$ structure (Figure 1B) is obtained in the presence of a dodecyltrimethylammonium chloride (DDAC) micelle folding scaffold at pH 5.6.¹⁶ The inhibitory $N\alpha$ state, with the β -sheet arrangement destabilized (Figure 1B), results at pH 7.4 in the presence of tetradecyltrimethylammonium chloride (TDAC) micelles, which contain two more methylene groups in the hydrocarbon tail and thus offers a larger, less curved surface.¹⁶ As discussed previously, these associations indicate that N is either bound to the CD or both the OMM and CD.¹⁶ Both conditions resulted in high-quality two-dimensional ^1H - ^{15}N correlation spectra for N_{M1-F50} of CPT1C indicative of successful sample preparations (Figure 2). Under the conditions promoting $N\beta$, peak pattern of N_{M1-H42} and N_{M1-F50} matched well up to Leu36 (Figure 2B). This residue resides within the last turn of helix $\alpha 2$ (Figure 1B) and consequently the spectral changes beyond Leu36 invariably arise from different peptide termini. In the $N\alpha$ state, only helix $\alpha 2$ is fully structured and identical between $N\alpha$ and $N\beta$ (Figure 1B),¹⁶ which implies changes between N_{M1-H42} and N_{M1-F50} to again localize to the helix $\alpha 2$ terminus. Thus, we proceeded exclusively with N_{M1-F50} .

The Non-inhibitory $N\beta$ State of CPT1C is Destabilized Relative to CPT1A

NMR spectroscopy provided residual dipolar coupling and backbone and side chain torsion angles structural restraints for simulated annealing calculations of the $N\beta$ structure of CPT1C. Out of an ensemble of 40 structures, the 20 lowest energy conformations were accepted and exhibited a coordinate precision of 1.42 Å for heavy atoms in the structured peptide region. Table I summarizes the structural statistics. The $N\beta$ structure revealed the N-terminal 23 residues to be dynamically unstructured (Figure 3A and 4B), i.e., the β -sheet arrangement found in CPT1A was not formed. Helix $\alpha 2$ was present and helical conformation extended beyond its C-terminal residue, Trp39, albeit with distortions (Figure 3B). The latter helical segment, termed helix $\alpha 2'$, encompasses Lys40-Trp47 (Figure 3A–B). The observed distortions could arise from adhering to the curved DDAC micelle surface. However, such distortions would be expected to be more uniform along helices $\alpha 2$ - $\alpha 2'$, suggesting that the distortion of $\alpha 2'$ arose mainly from optimizing the insertion of hydrophobic side chains in the micelle core. The distortion could aid the transition between N and the first TM helix.

To explain the absence of $\beta 1$ - $\beta 2$, secondary $^{13}C^\alpha$ shifts, which report on average backbone dihedral angles,¹⁷ were compared between $N\beta$ of CPT1C and CPT1A (Figure 4A). Except for the effects arising from the presence of helix $\alpha 2'$, shift differences localized to $\beta 1$ - $\beta 2$ with the swap in proline residues, stemming from Phe12Pro and Pro16Ser, exhibiting prominent effects. Pro residues disturb β -sheet conformation and the canonical hairpin sequence¹⁸ connecting $\beta 1$ - $\beta 2$ (Figure 1B) is destabilized by the substitution of Pro16. Low $\{^1H\}$ - ^{15}N NOE values, which correlate with H–N bond vector dynamics,¹⁹ for $\beta 1$ - $\beta 2$ relative to folded helices $\alpha 2$ - $\alpha 2'$ illustrated this destabilization further (Figure 4B). The Phe12Ser substitution in mouse CPT1C is milder (Figure 1C) but reduces the amphiphilic nature of $\beta 1$. Noteworthy is the substitution of Ala9 to glycine, which profoundly destabilizes $N\beta$ of CPT1A.¹⁶ Ala9 maintains packing interactions with Ala29 that stabilize

β 1- β 2 and orient the first eight amino acids away from the β -sheet (Figure 1B). The remaining amino acid substitutions within residues 9–24 of CPT1A and CPT1C (Figure 1C) appear innocuous in terms of β 1- β 2 stability. In conclusion, we interpret the Ala9Gly and Pro16Ser substitutions to purposefully destabilize the non-inhibitory N β state of CPT1C.

The Inhibitory N α State is Structurally Similar between CPT1C and CPT1A

In CPT1A, we inferred that MCoA occupies the substrate as well as the carnitine binding sites, and proposed N α to stabilize this blocked CD configuration.¹⁶ Compared to N β , the N α state of CPT1A loses the β 1- β 2 arrangement, maintains helix α 2 and develops propensity for another helix, termed α 1 (Figure 1B).¹⁶ However, in the presence of the micelle folding scaffold, a well-defined N α tertiary structure is not formed.¹⁶ Comparing secondary ¹³C α shifts between CPT1C and CPT1A, under the conditions that stabilize N α , showed that their secondary structure pattern matched (Figure 5A). Differences observed for β 1- β 2 arise from the swapped proline residues. However, in both cases, ¹³C α secondary shifts close to zero for residues that do not precede proline confirm a dynamically unstructured state. When comparing N α and N β of CPT1C directly (Figure 5B), the ¹³C α secondary shifts revealed that helix α 1 propensity was acquired in N α with little changes between both states in other segments. It is noteworthy that, despite destabilized β 1- β 2, the stability of helix α 1 still differed between N α and N β . At the pH value of the N β condition of 5.6, His5 is protonated, which perturbed the amphiphilic nature of helix α 1 (Ala2, His5, and Val8 line the apolar face, and Asp3, Gln6, and Ala7 constitute the polar helix face) and disfavored its micelle binding as shown by the absence of helical propensity in N β (Figure 5B). Thus, the stability of the inhibitory N α state is unchanged between CPT1C and CPT1A and the N α model of CPT1A (Figure 1B) serves to illustrate a possible backbone structure sampled by N α of CPT1C.

The conformation of helices α 2- α 2' in N α was essentially unchanged compared to N β of CPT1C (Figure 5B). Small changes in ¹³C α shifts at the border of helices α 2- α 2' may relate to the different intrinsic curvatures of the DDAC and TDAC micelles. Changes in the protonation state of His42 between the N α and N β solution conditions can contribute further. Finally, we specifically note the presence of Pro26 as second N-terminal residue of helix α 2 in CPT1C, which arose from a Glu26Pro substitution relative to CPT1A (Figure 1C). At this helix position, Pro is common^{20,21} and not disturbing helical geometry or hydrogen bonding. In N β of CPT1A, the side chain of Ser24 caps helix α 2, whereas the backbone conformation of Ser24 changes in CPT1C to orient its side chain away from a capping interaction which may be facilitated by Pro26 (Figure 3C). Notably, in CPT1A a Glu26-Lys561 electrostatic interaction has been implicated in inhibitory N α -CD interactions by complementary mutagenesis,²² which indicates that not only the N β -CD interplay has changed in CPT1C but also the N α -CD interaction. In sum, the structure of the inhibitory N α state is intact in CPT1C but its association with the CD appears to be altered compared to CPT1A.

DISCUSSION

CPT1C represents an enzyme for which limited biochemical information is available, especially regarding its regulatory mechanism. This circumstance relates to its apparent low catalytic activity in the presence of acyl-CoA substrates.^{6,7,10} Specifically, the low activity precludes measuring the concentration of MCoA at which half-maximal enzyme inhibition (IC₅₀ value) is observed.⁷ The correlation of IC₅₀ values with point mutations in N proved valuable for establishing the regulatory mechanism of CPT1A.¹⁶ Consequently, rather than explaining function by structure, the obtained structural information can only be used to identify and discuss a number of possible CPT1C regulatory scenarios. We start by evaluating the implications of destabilizing the β 1- β 2 arrangement (residues 9–24) in

CPT1A for which pertinent IC_{50} values are available. In CPT1A, Ala9Gly lowers the IC_{50} value approximately 17 times.¹⁶ The effects of destabilizing the $\beta 1$ - $\beta 2$ hairpin in N β of CPT1C can be represented by Gly18Ala in CPT1A, which reduces IC_{50} by a factor of approximately 3.¹⁶ In CPT1A, the combination of these two mutations would increase the N α :N β ratio and heighten its MCoA sensitivity by a factor of up to 51. In the presence of MCoA, this would put the catalytic activity of CPT1A close to the activity observed for CPT1C. Because CPT1C binds MCoA with a similar affinity as CPT1A,^{6,7,10} we expect it to be sensitive to MCoA inhibition and form an inhibitory N α -MCoA-CD complex. However, in CPT1C activity assays, no external MCoA was added. Assays used CPT1C-containing membrane fractions of lysed cells.^{6,7,10} Mammalian cells contain low micromolar concentrations of MCoA,⁵ but it is unclear if endogenous MCoA, in particular its ester linkage, is sufficiently stable in cell extracts to impact the assays. Regardless of the basal acyl-CoA transfer activity of CPT1C or its precise substrate identity, the structural properties of N suggest an elevated MCoA sensitivity compared to CPT1A, perhaps in relation to a sensory instead of catabolic function in neurons.

The extensive destabilization of the N β state in CPT1C implies an infinite N α :N β ratio and, thus, the virtual absence of a non-inhibitory N state. In CPT1A, the non-inhibitory N β state comes into being by associating with the CD and, through the N α :N β ratio, allows the enzyme to sense the long-term metabolic state that is encoded in the OMM properties.¹⁶ At first sight, in relation to the ER membrane this functionality appears to be lost in CPT1C. However, in analogy to the stabilization of N β by the CD of CPT1A, another protein may interact with N in a non-inhibitory fashion. A regulatory protein could use N as a ligand and induce a non-inhibitory state N β^* . Such a protein, termed R, would lower the proportion of N α and could even activate the CD directly, thus forming an active N β^* · R-CD complex and again assigning functionality to the N α :N β^* ratio.

MATERIALS AND METHODS

Expression and Purification of N-terminal CPT1C Peptides

Met1-Phe50 of human CPT1C was synthesized from overlapping oligonucleotides²³ and cloned into the pET-44 expression vector with the third IgG-binding domain of Protein G (GB3) as N-terminal fusion protein and an intervening tobacco etch virus (TEV) protease cleavage site. A shorter peptide, encompassing Met1-His42, which corresponds to the peptide length studied for N of CPT1A,¹⁶ was prepared by introducing a stop codon for residue 43. Gene expression was induced in *E. coli* BL21(DE3)pLysS, T1^R cells (Sigma-Aldrich, Inc.) growing in M9 minimal medium at 37 °C by adding IPTG to 1.0 mM. Isotope labeling was achieved by supplying 99% ¹³C-D glucose, 99% ¹⁵NH₄Cl and 99% D₂O. Following cell lysis by sonication in 50 mM NaH₂PO₄/Na₂HPO₄, pH 7.4, 300 mM NaCl, 100 mM SDS, 20 mM imidazole, 2 mM β -mercaptoethanol solution, the fusion protein was purified by immobilized metal affinity chromatography (IMAC) using a HiTrap IMAC HP column (GE Amersham, Inc.) charged with Ni²⁺. The column was washed with 50 mM NaH₂PO₄/Na₂HPO₄, pH 7.4, 300 mM NaCl, 25 mM SDS and the bound fusion protein was eluted in 50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 8 M urea, 300 mM imidazole. The eluted fraction was dialyzed against 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 100 mM NaCl and the N peptide was cleaved from the fusion protein using TEV protease at a molar ratio of 1:50 in the presence of 1 mM DTT overnight at 30 °C. The fusion protein and any remaining uncleaved protein were removed by IMAC. The N peptide was further purified by HPLC using a Hamilton PRP-3 reverse-phase column. A linear gradient, ranging from 70%/30% buffer A (H₂O, 0.1% TFA) / buffer B (80% acetonitrile, 0.1% TFA) to 25%/75% in 40 min was employed. Peptide purity was verified by SDS-PAGE and NMR subsequent to freeze-drying.

NMR Sample Preparation

N peptide concentration was measured in acetonitrile-water solution by UV spectroscopy using $\epsilon_{280\text{nm}}(\text{N}_{\text{M1-F50}}) = 12,490 \text{ M}^{-1}\text{cm}^{-1}$ and $\epsilon_{280\text{nm}}(\text{N}_{\text{M1-H42}}) = 6,990 \text{ M}^{-1}\text{cm}^{-1}$. Defined amount of freeze-dried peptide was taken up in a volume of 280 μl to yield sample N β , containing 0.5 mM N, 25 mM MES-NaOH (pH 5.6), 150 mM dodecyltrimethylammonium chloride (DDAC), and sample N α , containing 0.25 mM N, 25 mM HEPES-NaOH (pH 7.4), 100 mM tetradecyltrimethylammonium chloride (TDAC). The N peptide-DDAC micelle complex was aligned relative to the magnetic field by stretched, negatively charged polyacrylamide gel of 320 μl volume. The gel was polymerized from a 5.2% w/v solution of acrylamide (AA), 2-acrylamido-2-methyl-1-propanesulfonate (AMPS) and bisacrylamide (BIS), with a monomer-to-crosslinker ratio of 39:1 (w/w) and a molar ratio of 96:4 of AA to AMPS.²⁴ Subsequent to transferring the gel into an open-ended NMR tube, a ^2H splitting of 5.0 Hz was observed.

NMR Spectroscopy and Structure Calculation

NMR data was acquired using $^2\text{H}/^{13}\text{C}/^{15}\text{N}$ -labeled N peptides on a cryoprobe-equipped Bruker Avance 700 spectrometer at 35 °C. HNCA, HNCACB and HNCO experiments were performed to achieve backbone assignments. HNCO-based experiments were employed for the measurement of $^3J_{\text{C}'\text{C}\gamma}$ and $^3J_{\text{NC}\gamma}$ couplings,²⁵ and the detection of $^1J_{\text{NH}}$, $^1J_{\text{CaC}'}$, $^1J_{\text{C}'\text{N}}$ as well as $^1J_{\text{NH}}+^1D_{\text{NH}}$, $^1J_{\text{CaC}'}+^1D_{\text{CaC}'}$, $^1J_{\text{C}'\text{N}}+^1D_{\text{C}'\text{N}}$ couplings^{26–28} in isotropic or aligned samples. $\{^1\text{H}\}$ - ^{15}N NOE measurements were performed with 5 s of presaturation preceded by a recycling delay of 4 s for the NOE experiment and a 9 s recycle delay for the reference experiment. TROSY-type H–N detection²⁹ was used in all experiments. The structure of the well-folded Leu23-Trp47 residues of N $_{\text{M1-F50}}$ in complex with a DDAC micelle (Figure 4B) was calculated by simulated annealing, starting at 3000 K using the program XPLOR-NIH.³⁰ Backbone dihedral angle constraints were obtained from the pattern of N, $\text{H}^{\alpha\text{C}\alpha}$, C^{β} and C' chemical shifts.³¹ $^3J_{\text{C}'\text{C}\gamma}$ and $^3J_{\text{NC}\gamma}$ coupling constants instructed χ_1 side-chain angle restraints (Table S1). Aside from standard force field terms for covalent geometry (bonds, angles, and improper dihedrals) and nonbonded contacts (Van der Waals repulsion), dihedral angle restraints were implemented using quadratic square-well potentials. In addition, a backbone-backbone hydrogen-bonding potential and torsion angle potential of mean force were employed.^{32,33} A quadratic harmonic potential was used to minimize the difference between predicted and experimental residual dipolar couplings (RDC; Δ^2D). The final values for the force constants of the different terms in the simulated annealing target function were as follows: 1,000 kcal·mol^{−1}·Å^{−2} for bond lengths, 500 kcal·mol^{−1}·rad^{−2} for angles and improper dihedrals, 4 kcal·mol^{−1}·Å^{−4} for the quartic Van der Waals repulsion term, 500 kcal·mol^{−1}·rad^{−2} for dihedral angle restraints, 0.3 kcal·mol^{−1}·Hz^{−2} for $^1D_{\text{NH}}$ RDC restraints with $^1D_{\text{C}'\text{N}}$ and $^1D_{\text{CaC}'}$ scaled relative to $^1D_{\text{NH}}$ corresponding to their dipolar interaction constants, 1.0 for the torsion angle potential, and a directionality force of 0.20 and a linearity force of 0.05 for the hydrogen-bonding potential. A total of 40 structures were calculated of which the 20 lowest energy structures were accepted. Table I reports structural statistics. Coordinates and structural constraints have been deposited under Protein Data Bank ID 2m76 and Biological Magnetic Resonance Bank ID 19174.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIGURE 1.

Overview of carnitine palmitoyltransferase 1 enzymes. **(A)** Structural model of the human CPT1A enzyme.¹⁶ The structure of the CPT1A regulatory domain, termed N, in the non-inhibitory N β state (PDB ID 2LE3) is shown in complex with modeled transmembrane and catalytic domains, termed TM1/TM2 and CD, respectively. **(B)** A model of the N α state and the structure of the N β state of human CPT1A are depicted in cartoon representation. Amino acids that are substituted in CPT1C are shown in stick representation. **(C)** Sequence alignment of N for the three mammalian CPT1 isoforms. Conserved amino acids are colored by the Jalview multiple alignment editor³⁴ using the ClustalX color scheme.

FIGURE 2.

^1H - ^{15}N correlation NMR spectra of $\text{N}\alpha$ and $\text{N}\beta$ of human CPT1C. (A) Spectrum with shown backbone assignments of the $^2\text{H}/^{13}\text{C}/^{15}\text{N}$ -labeled $\text{N}_{\text{M1-F50}}$ peptide in complex with a TDAC micelle at pH 7.4, resulting in the $\text{N}\alpha$ state. The spectrum was recorded at peptide and TDAC concentrations of 0.25 and 100 mM, respectively, at 25 °C and a ^1H frequency of 700 MHz. (B) Spectra of ^{15}N -labeled $\text{N}_{\text{M1-F50}}$ and $\text{N}_{\text{M1-H42}}$ peptides in complex with DDAC micelles at pH 5.6, yielding the $\text{N}\beta$ state. Backbone assignments of $\text{N}_{\text{M1-F50}}$ are shown and, up to Leu36, closely match $\text{N}_{\text{M1-H42}}$ peak positions. Spectra are shown at identical contour levels and were recorded using peptide and DDAC concentrations of 0.1 mM and 50 mM, respectively, at 35 °C and a ^1H frequency of 700 MHz.

FIGURE 3.

Structure of the N β state of human CPT1C. Ensemble of 20 calculated simulated annealing structures, showing the backbone in *red* and side chains in *cyan*. The structures were superimposed on the heavy atom backbone coordinates of the well-structured Leu23-Trp47 residues. The folded nature of helices $\alpha 2$ - $\alpha 2'$ contrasts the dynamically unstructured conformations of residues Met1-Glu22. **(B)** Cartoon representations of the lowest energy ensemble structure. The polar and apolar faces of helices $\alpha 2$ - $\alpha 2'$ are oriented toward the top and bottom of the page, respectively. The shown orientations are related by a rotation of 180° about the y-axis. **(C)** Illustration of differences in N-terminal helix $\alpha 2$ capping between CPT1C and CPT1A. Ncap denotes the capping residue Ser24 and N1 to N4 represent the first four residues of the helix. The H^N atoms of N2-N4 are shown in violet.

FIGURE 4.

Comparison of secondary structure and backbone dynamic properties of N β of CPT1C and CPT1A. **(A)** Secondary $^{13}\text{C}^\alpha$ chemical shifts, $\Delta\delta(^{13}\text{C}^\alpha)$, defined as the difference between experimental and tabulated, random coil $^{13}\text{C}^\alpha$ shifts, correlate with backbone conformation.¹⁷ For $^2\text{H}/^{13}\text{C}/^{15}\text{N}$ -labeled N, random coil conformations are obtained at approximately -0.5 ppm, whereas positive and negative shifts relative to this value denote helical and extended backbone propensities, respectively.¹⁷ **(B)** $\{^1\text{H}\}\text{-}^{15}\text{N}$ NOE values correlate with H–N bond vector dynamics¹⁹ and illustrated the dynamically unstructured nature of $\beta 1\text{-}\beta 2$.

FIGURE 5.

Comparison of secondary structure properties of N α between CPT1C and CPT1A and between N α and N β of CPT1C. Secondary structure propensities are compared in the form of secondary $^{13}\text{C}^\alpha$ chemical shifts, $\Delta\delta(^{13}\text{C}^\alpha)$.¹⁷

Table I Structural statistics for the N β structure of CPT1C^a

R.m.s. deviations from experimental dihedral restraints (deg)	
All (52) ^b	1.58 $\pm$ 0.31
R.m.s. deviations from experimental residual dipolar couplings (Hz) ^c	
¹ D _{NH} (24)	2.02 $\pm$ 0.77
¹ D _{NC'} (24)	2.11 $\pm$ 1.00
¹ D _{CaC'} (23)	1.99 $\pm$ 0.84
Deviations from idealized covalent geometry	
Bonds (Å)	0.003 $\pm$ 0.000
Angles (deg)	0.528 $\pm$ 0.019
Impropers (deg)	0.440 $\pm$ 0.031
Coordinate precision (Å) ^d	
Backbone non-hydrogen atoms	0.53
All non-hydrogen atoms	1.41
Measures of structural quality	
E _{LJ} (kcal mol ⁻¹) ^{3 e}	-161.1
Residues in most favorable region of Ramachandran plot ^f	95.9%

^aStatistics for residues Leu23-Trp47 for the 20 lowest energy structure out of 40 calculated simulated annealing structures.^bTorsion angle restraints included 25 ϕ , 25 ψ , and 2 χ_1 angles.^cR.m.s. deviations are normalized to an alignment tensor magnitude of 10 Hz.^dDefined as the average r.m.s. difference between the 20 accepted simulated annealing structures and the mean coordinates.^eThe Lennard-Jones van der Waals energy was calculated with the CHARMM PARAM 19/20 parameters and was not included in the simulated annealing target function.^fCalculated using PROCHECK V3.4.4.