Crystal Structure of an \(L\)-Carnitine Complex with Pyrogallol[4]arene

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Abstract. \(L\)-Carnitine is essential for the transport of long-chain fatty acids from cytosol into mitochondria for generating metabolic energy. The survey of crystal structures of carnitine-containing proteins in the Protein Data Bank reveals that carnitine can take several conformations with the quarternary trimethylammonium terminal being always bound to aromatic residues through cation–\(\pi\) interactions in acyltransferases or carnitine-binding proteins. In order to demonstrate the importance of cation–\(\pi\) interaction as a carnitine recognition mechanism in the artificial receptor–ligand system that mimics the carnitine-binding sites, we have determined the crystal structure of a complex formed between \(L\)-carnitine and pyrogallol[4]arene (pyrogallol cyclic tetramer: PCT) as a carnitine receptor, \(2\text{PCT} \cdot 2(L\text{-carnitine}) \cdot 4\text{EtOH}\). There form two crystallographically independent monomeric \([\text{PCT}\cdot L\text{-carnitine}]\) substructures, which further form an obliquely arranged capsule-like dimeric \([\text{PCT}\cdot L\text{-carnitine}]_2\) structure through a pair of O–H (PCT)–O (\(L\text{-carnitine}\)) hydrogen bonds. This is the first report of PCT complex with chiral molecules. In each of the two monomeric \([\text{PCT}\cdot L\text{-carnitine}]\) substructures, the \(L\)-carnitine molecule takes the elongated form with an intramolecular hydrogen bond between the hydroxyl group and the carboxylate oxygen, and the cationic trimethylammonium moiety is incorporated into the cavity of the bowl-shaped PCT molecule through cation–\(\pi\) interactions. These features are similar to those at the \(D\)-carnitine-binding site in the crystal structure of the glycine betaine/carnitine/choline-binding protein complex.

1. Introduction

Carnitine is needed for the transport of long-chain fatty acids through mitochondrial membrane; long-chain acyl-CoA synthesized in cytosol can’t be transported as such into mitochondria but must be once converted to acyl-carnitine by carnitineacyltransferase I using carnitine, and incorporated acyl-carnitine in mitochondria is subsequently catalyzed by carnitineacyltransferase II to give acyl-CoA again, followed by \(\beta\)-oxidation [1, 2]. Because \(\beta\)-oxidation in mitochondria is an important metabolism pathway of fatty acids, the research of the molecules targeting acyltransferases is proceeded in expectation of the application for diet foods or supplements.
Crystal structures of three kinds of protein–carnitine complexes are reported in the Protein Data Bank. In the acetyltransferase–l-carnitine complex [3], the carboxylate group of the l-carnitine ligand forms hydrogen bonds with tyrosine, serine and threonine residues in the protein, the hydroxyl group forms a hydrogen bond with the histidine residue, and the quaternary trimethylammonium group forms a cation–π interaction with the phenylalanine residue, stabilizing the bent structure of the carnitine molecule. In the glycine betaine/carnitine/choline-binding protein–D-carnitine complex [4], the carboxylate group of D-carnitine forms hydrogen bonds with glutamine and asparagine residues in the protein and the trimethylammonium cation forms cation–π interactions with surrounding four tyrosine residues, stabilizing the elongated structure of carnitine with an intramolecular hydrogen bond formed between the hydroxyl group and the carboxylate oxygen. The carnitine molecule takes different conformations depending on each binding protein, but in all cases, the cation–π interaction between the trimethylammonium cation in carnitine molecule and aromatic rings in proteins (C⋯ aromatic ring < 4.1 Å) is observed. This suggests the common importance of the cation–π interaction in the carnitine–protein recognition and binding.

In order to demonstrate the importance of the cation–π interaction as a ligand recognition mechanism, we have constructed artificial receptor–ligand systems that mimic interactions at ligand-binding sites between the quaternary alkylammonium moiety of the bioactive ligands, involving acetylcholine, and the aromatic ring(s) of the proteins [5-9]. Most recently, as such a system, we have reported [10] crystal structures of the complexes formed between resorcin[4]arene or tetramethylated resorcin[4]arene and l-carnitine. We report here the crystal structure of an l-carnitine complex with pyrogallol[4]arene (PCT) as a carnitine receptor, showing that cation–π interaction formed between the trimethylammonium moiety of l-carnitine and aromatic rings of PCT and that the l-carnitine conformation is similar to that in the glycine betaine/carnitine/choline-binding protein complex. Seven structures of PCT complexes have already been reported, but all have an inversion center, and consist of only achiral molecules. The present structure has no inversion center, because chiral l-carnitine is included.

2. Materials and Methods
PCT (1) host compound was synthesized by an analogous method [11] for RCT. The host solution and a l-carnitine solution were prepared by dissolving the host compound (700 mg) in ethanol (42 mL) and l-carnitine (1.0 g) in water (10 mL), respectively. The host solution (0.1 mL), l-carnitine solution (2 mL) and water (0.4 mL) were mixed and allowed to stand at room temperature to give dark-orange crystals after 5 days. Molecular formula was determined by X-ray analysis.

X-ray intensity data were measured on a Rigaku AFC-7R diffractometer (at the Instrument Center of the Institute for Molecular Science in Okazaki) with a rotating anode generator and a Mercury CCD camera, using graphite-monochromated Mo Kα radiation (λ(Mo Kα) = 0.7169 Å) at 295 K. Data reduction, the cell refinement, and semi-empirical absorption corrections were performed with the program CrystalClear [12]. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F², minimizing Σw( |Fo| - |Fc|)², with the programs SHELXS and SHELXL [13], respectively, on the platform and graphic software Yadokari-XG [14]. No attempt was made to locate H atoms. Crystal data for the PCT complex, 2(1)-2(L-carnitine)-4EtOH: C₉₆H₁₄₂N₂O₃₄, fw = 1834.04, triclinic, space group P1, a = 12.7778(9), b = 12.9479(10), c = 15.0200(15) Å, α = 89.832(7), β = 82.108(5), γ = 69.720(4)°, V = 3426.62(3) Å³, Z = 1, Dcalcd = 1.240 g cm⁻³, μ(Mo Kα) = 0.149 cm⁻¹, F(000) = 914. All non-H atoms were refined with anisotropic temperature factors. Final R = 0.089 and Rw = 0.230 (for 10318 reflections with I > 2σ(I) out of 14100 unique reflections in the range 2 < 2θ < 55°) and GOF = 1.55. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-809923. Copies of the data can be obtained free of charge from the Cambridge, CB2 1EZ, UK; Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk.
3. Results and Discussion

In the asymmetric unit, two PCT (PCT1 and PCT2), two carnitine (Car1 and Car2) and four solvent ethanol molecules are included. As shown in Figure 1, there form two crystallographically independent monomeric PCT-l-carnitine substructures, [PCT1-Car1] and [PCT2-Car2], which further form an obliquely arranged [PCT-l-carnitine]2 dimeric structure through an inter-substructure O–H (PCT1)···O (Car2) hydrogen bond (O(1)···O(28) = 2.951(8) Å). In each of the two substructures, the cationic trimethylammonium end of the carnitine molecule is incorporated into the cavity of a bowl-shaped PCT molecule through cation–π interactions. In addition, in the [PCT1-Car1] substructure, the carboxylate end of Car1 forms an intra-substructure hydrogen bond with the phenolic oxygen of PCT1 (O(10)···O(25) = 2.908(8) Å). Car1 and Car2 ligands take a fully extended backbone (N–C–C–C–C) conformation with an intramolecular O–H (hydroxyl)···O (carboxylate) hydrogen bond (O(27)···O(25) = 2.881(7) Å for Car1 and O(30)···O(29) = 2.667(7) Å for Car2). These observations are in consistent with a thermodynamic solution study [15] showing that l-carnitine was strongly bound to PCT with $K = 18,000 \text{ M}^{-1}$ and all of the functional groups (trimethylammonium, carboxylate, and hydroxyl) and the extended hydrocarbon chain are assumed to contribute to the affinity enhancement through cation–π, hydrogen bonding, ion-dipole, and van der Waals interactions (see Figure 2 in Ref. 15).

![Figure 1. Molecular structure of the [l-carnitine]2 dimer. Distances (Å) from the carbon atoms of the trimethylammonium group of carnitine to the π-centroids of 1 (< 4.1 Å): for Car1–PCT1, C78···ring A = 3.72, C77···ring B = 3.76, C78···ring C = 3.63, C78···ring D = 3.63; for Car2–PCT2, C86···ring E = 3.62, C86···ring F = 3.74, C86···ring G = 3.63, and C84···ring H = 3.76. Broken lines denote hydrogen bonds.]

All crystal structures of PCT complexes registered in Cambridge Structural Database have a crystallographic inversion center which generates upside-down arranged two PCT molecules in the crystal lattice to construct a capsule or a capsule-like supramolecule. The present PCT complex has no inversion center because the chiral l-carnitine molecule is included but, interestingly, a capsular-like supramolecule is formed here in a similar manner to the known PCT complex structures. As shown in Figure 2, though the whole backbone shapes in the two substructures are similar to each other, each hydrogen bond network is not the same. Closer observations reveal that carboxylate of
Car1 forms hydrogen bonds to three PCT molecules, including the pairing PCT molecule, while carboxyl group of Car2 forms hydrogen bonds to three PCT molecules, not including the pairing PCT, but including the opposite side substructure’s PCT molecule.

Figure 2. Crystal packing of the [L-carnitine]₂ dimer viewed from α-axis direction of the unit cell. PCT, carnitine and ethanol molecules construct hydrogen bonding networks expanding laterally, which layers pile up, forming the hydrophobic layers. Light blue lines denote hydrogen bonds.

Though the complex was prepared under a mixture of water and ethanol, only ethanol molecules were located in the crystal structure. Oxygen atoms of all the four ethanol molecules construct hydrogen bonding networks with PCT molecules, and the methyl groups direct toward hydrophobic layers made by lateral array of PCT molecules, where two ethanol molecules have C–H···π interactions in the distance less than 4.1 Å with aromatic rings of PCT. These well fitted ethanol molecules may contribute to the stabilization of the crystal structure and a small R-factor, 8.9%, compared with resorcin[4]arene or tetramethylated resorcin[4]arene complexes with carnitine, which showed higher R values, 11.7 or 13.1%, respectively [10].

Figure 3. The d-carnitine ligand and the close contact residues in the glycine betaine/carnitine/choline-binding protein complex structure. The distances of hydrogen bonds (< 3.0 Å) and cation–π interactions (< 4.1 Å) are denoted.
The carbon atoms of the trimethylammonium moiety of Car1 and Car2 molecules are in distances to form cation–π interactions to aromatic rings of PCT1 and PCT2, as noted above (Figure 1). Also in the structure of the glycine betaine/carnitine/choline-binding protein complex [4], aromatic rings of four tyrosine residues surround the trimethylammonium end of carnitine (Figure 3). Differences around trimethylammonium cation between the present complex and the protein complex are angles between a carnitine molecule and four aromatic rings, and the scale of the cavity made by four aromatic rings. The angles between the vector made of a carbon atom of carboxylate and a nitrogen atom and the plane comprised of bottom carbon atoms of four aromatic rings of the PCT complex and the protein complex are almost perpendicular and about 20°, respectively. The distances between the two aromatic rings which are opposite to each other at the upper part of the cavity are 8–9 Å for both the PCT complex and the protein complex, while at the bottom part of the cavity, the corresponding distances are about 5 and 8–9 Å, respectively. The bottom of the PCT molecule is too narrow for the carnitine molecule to insert enough its cationic end into the cavity, resulting in the different spatial arrangement of cation–π interactions, comparing with that in the protein complex. To reach the precise imitation of the carnitine binding structure and to design more appropriate carnitine-receptor molecules that mimic the carnitine binding site in the glycine betaine/carnitine/choline-binding protein, these points should be improved utilizing different-shaped synthetic receptors [16] which have linkers with different length. The carboxylate oxygen atoms of Car1 and Car2 form hydrogen bonds to three PCT molecules and Car1 and Car2 have intramolecular hydrogen bonds. These interactions stabilize the elongated structure of Car1 and Car2, which is similar to that of the glycine betaine/carnitine/choline-binding protein complex but different from the partially folded structure in the RCT–L-carnitine complex [10] (Figure 4).

4. Conclusion
This study provides an additional X-ray example that shows the existence of cation–π interaction between the quaternary trimethylammonium moiety of the biologically important L-carnitine ligand and aromatic rings in the artificial receptor–ligand system that mimics the carnitine binding sites in
acyltransferases or carnitine-transporters. The extended structures of carnitine ligands are similar to that observed in the glycine betaine/carnitine/choline-binding protein complex [4].

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