ACETYLCARNITINE: ON THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION

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Acetylcarnitine chloride, a molecule with cholinergic properties, has been studied by X-ray crystallographic techniques. Results show that a portion of the acetylcarnitine molecule is in the same configuration as the functionally similar acetylcholine molecule and other cholinergic molecules.

INTRODUCTION

Carnitine and its derivatives have been found in a large number of plant and animal tissues.[1,2] It is now believed that carnitine functions as a carrier of fatty acids into mitochondria.[3] However, carnitine is found in large quantities in the flight muscles of flies which metabolize carbohydrates rather than fatty acids during flight.[4] Large increases of acetylcarnitine concentration during the flight of the blowfly (Phormia reginia) and the presence of an active acetylcarnitine transferase indicate that acetylcarnitine is important in carbohydrate metabolism.[4] More recent work has shown that phospholipid membranes are permeable to palmitoylcarnitine but not to acetylcarnitine.[3] These data have led several workers to suggest that acetylcarnitine serves as a reservoir for high-energy acetyl groups.[3,4]

Acetylcarnitine has also been shown to have powerful cholinergic properties[5,6] and appears to have pharmacologic properties similar to acetylcholine.[2] Acetylcarnitine has been synthesized by the choline acetylase system isolated from brain tissue and is destroyed by cholinesterase.[7] Experiments also indicate that choline is an alternate substrate for carnitine acetyltransferase.[8] We have undertaken the crystallographic study of acetylcarnitine in order to investigate the relationship between molecular configuration and cholinergic function.

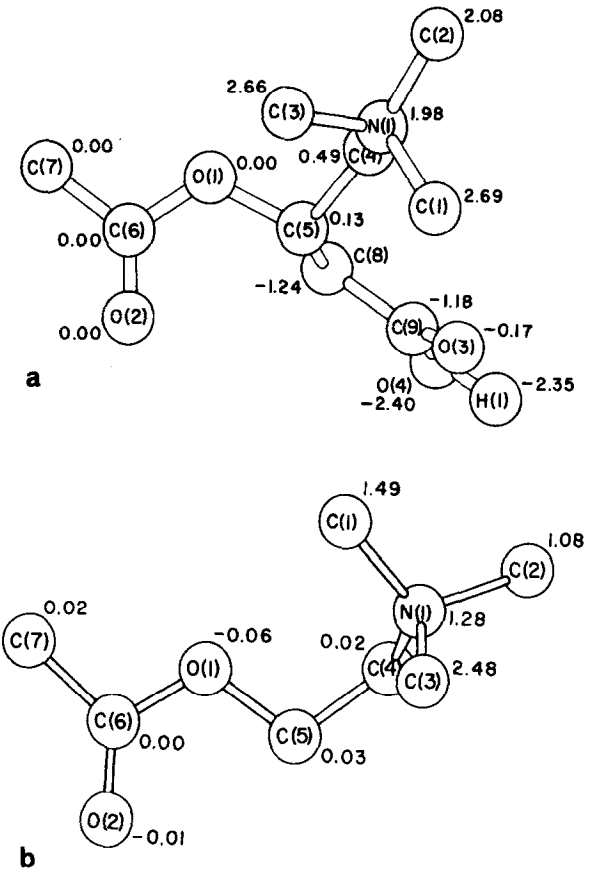

Figure 1a. The conformation of acetyl dl-carnitine ion in crystals of acetyl dl-carnitine chloride.

Figure 1b. For comparison, the conformation of acetylcholine in crystals of acetylcholine perchlorate is shown.

EXPERIMENTAL

The compound acetyl dl-carnitine chloride was prepared by "method A" of Ziegler, Bruckner and Binon.[9] Single crystals were obtained by allowing diethyl ether to slowly diffuse into saturated solutions of acetyl dl-carnitine chloride at 2°C. The flat plates that resulted were cleaved into needle-like crystals suitable for x-ray diffraction study. The crystals are monoclinic (space group $P2_1/C$) with unit cell dimensions, a = 6.390 Å, b = 13.34 Å,

TABLE 1

POSITIONAL PARAMETERS

Atom	x	y	z
Cl(1)	0.73849	0.59693	0.35099
N(1)	0.84819	0.65141	0.62089
O(1)	0.79780	0.56280	0.82195
O(2)	1.00001	0.44396	0.88363
O(3)	1.35516	0.71811	0.75295
O(4)	1.36547	0.77930	0.89435
C(1)	1.05224	0.64040	0.57066
C(2)	0.71667	0.72841	0.57313
C(3)	0.73509	0.55241	0.62006
C(4)	0.88220	0.69062	0.71703
C(5)	0.97721	0.61691	0.78357
C(6)	0.82953	0.47630	0.86757
C(7)	0.62824	0.42947	0.89227
C(8)	1.08436	0.67438	0.85986
C(9)	1.28230	0.72490	0.82824
H(1,1)	1.11968	0.70559	0.56735
H(1,2)	1.13993	0.58800	0.60309
H(1,3)	1.02517	0.62736	0.50363
H(2,1)	0.57596	0.72628	0.60830
H(2,2)	0.69079	0.70376	0.50883
H(2,3)	0.78707	0.79557	0.57346
H(3,1)	0.59293	0.56516	0.65752
H(3,2)	0.81662	0.50065	0.64852
H(3,3)	0.69292	0.53540	0.55071
H(4,1)	0.98171	0.75006	0.70893
H(4,2)	0.72574	0.71012	0.73878
H(5,1)	1.08767	0.56635	0.74944
H(7,1)	0.52083	0.48105	0.90358
H(7,2)	0.62849	0.38391	0.94480
H(7,3)	0.57666	0.39834	0.83582
H(8,1)	1.11898	0.62583	0.91098
H(8,2)	0.98837	0.72617	0.89057
H(1)	1.49613	0.81379	0.87228

c = 14.800 Å and β = 90.17°. Three-dimensional intensity data were collected on a Phillips PAILRED diffractometer employing equi-inclination geometry and using silicon (111) monochromatized molybdenum K_α radiation. A fixed counter moving crystal scan was used, with a scan rate of 0.5° per minute and scan ranges of 2.0° to 3.0°. Background radiation was counted at the beginning and end of the scan for 120 to 200 seconds. All reflections for which the statistical counting error exceeded 50% were rejected.[10] A total of 4234 reflections were measured. Of these, 1616 independent reflections were considered to be

TABLE 2

BOND LENGTHS

Atoms	Distance in Angstroms
C(1) - N(1)	1.51
C(2) - N(1)	1.50
C(3) - N(1)	1.51
N(1) - C(4)	1.53
C(4) - C(5)	1.52
C(5) - O(1)	1.47
O(1) - C(6)	1.35
C(6) - O(2)	1.19
C(6) - C(7)	1.48
C(5) - C(8)	1.53
C(8) - C(9)	1.51
C(9) - O(3)	1.21
C(9) - O(4)	1.33
O(4) - H(1)	1.01

BOND ANGLES

Apex	End	End	Angle in Degrees
N(1)	C(1)	C(2)	109
N(1)	C(1)	C(3)	109
N(1)	C(2)	C(3)	109
N(1)	C(1)	C(4)	112
N(1)	C(2)	C(4)	106
N(1)	C(3)	C(4)	112
C(4)	N(1)	C(5)	116
C(5)	C(4)	O(1)	105
C(5)	C(4)	C(8)	109
C(5)	O(1)	C(8)	108
O(1)	C(5)	C(6)	120
C(6)	O(1)	O(2)	123
C(6)	C(7)	O(2)	126
C(6)	C(7)	O(1)	111
C(8)	C(5)	C(9)	112
C(9)	C(8)	O(3)	125
C(9)	O(3)	O(4)	124
C(9)	C(8)	O(4)	110
O(4)	C(9)	H(1)	110

observed and used in the analysis. The X-RAY SYSTEM of computer programs was used to analyze these data.[11]

The chlorine atom positions were located from a three-dimensional Patterson synthesis. The chlorine atom was then used as a phasing model and

twelve additional atoms were located from a three-dimensional Fourier synthesis. The remaining non-hydrogen atom positions were obtained from a second three-dimensional Fourier synthesis. Positional parameters and isotropic temperature factors were refined to an R value 12.9%. Hydrogen atom positions were then located from a three-dimensional Fourier difference synthesis. Refinement was continued on all positional parameters and on anisotropic temperature factors for the non-hydrogen atoms. Temperature factors for the hydrogen atoms were set at U = 0.076 where $U = B/8\pi^2$.[12] The final value of the conventional R factor was 6.4%. Final positional parameters for all atoms are listed in Table 1.

RESULTS AND DISCUSSION

The conformation of the acetylcarnitine molecule is shown in Figure 1a. The distance in angstroms of each atom from the plane formed by C(7), C(6), O(1) and O(2) is given. Table 2 lists the calculated bond lengths and bond angles for the non-hydrogen atoms and the acid hydrogen. The estimated standard deviations are approximately ± 0.007 Å for the bond lengths and approximately ± 0.4° for the bond angles. A hydrogen bond exists between chlorine and H(1). The bond length Cl(1) - H(1) is 1.98 Å, and the bond angle O(4) - H(1) - Cl(1) is 166°. The O(4) - Cl(1) distance of 2.97 Å is smaller than the reported value of 3.04 Å for choline chloride.[13] Each chlorine is surrounded by nitrogen placed at the corners of a distorted tetrahedron at distances ranging from 4.13 to 5.02 Å.

Acetylcarnitine can be thought of as the β-carboxymethyl derivative of acetylcholine. As might be expected from the cholinergic properties of acetylcarnitine, the acetylcholine segment is in a conformation equivalent to that previously reported for acetylcholine.[14,15] Figure 1b shows the conformation of acetylcholine in crystals of acetylcholine perchlorate[15] for comparison. The distance in angstroms of each atom from the plane formed by C(7), C(6), O(1), O(2), C(5) and C(4) is given. Table 3 lists torsional

TABLE 3

Selected Torsional Angles for Some Cholinergic Molecules

Molecule	C(2)-N(1)-C(4)-C(5)	N(1)-C(4)-C(5)-O(1)	C(4)-C(5)-O(1)-C(6)	C(5)-O(1)-C(6)-O(2)
Acetylcholine Bromide[16]	180°	77°	79°	0°
Acetylcholine Chloride[14]	171.4°	84.7°	-166.9°	5.2°
Acetylcholine Perchlorate[15]	168.3°	73.7°	179.8°	0.8°
Acetylcarnitine Chloride	-169.2°	87.9°	-164.5°	-5.3°
Acetyl-α-Methylcholine[17]	--	90°	170°	--
L-(+)-acetyl-β-methylcholine iodide[18]	--	85°	-147°	--
L-Muscarine Iodide[19]	--	85°	157°	--
L-Lactoylcholine Iodide[20]	--	73°	144°	--

angles for some cholinergic molecules and for acetylcarnitine. The average N(1) - O(1) distance for the three acetylcholine structures is 3.23 Å and that distance is 3.22 Å for acetylcarnitine. The distance from O(1) to the nearest nitrogen methyl group averages 3.06 Å for acetylcholine and is 3.02 Å for acetylcarnitine. The other four molecules listed in Table 3 are in the same configuration. The structural similarity of these cholinergic molecules indicates the strong correlation that exists between structure and function in this system.

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