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# Crystal Structures of Carnitine and Acetylcarnitine Zwitterions: A Structural Hypothesis for Mode of Action

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The solid-state structures of the zwitterionic forms of both carnitine and acetylcarnitine have been determined by single-crystal X-ray analysis. The crystal structure of acetylcarnitine reveals a different backbone conformation from that of carnitine. The conformational differences observed for carnitine and acetylcarnitine are more a consequence of steric than electrostatic effects. A detailed comparison is made between the zwitterionic structures and previously published hydrochloride salts. The effect of charge distribution on conformation is discussed. The zwitterionic structures do not exhibit enhanced electrostatic attraction between carboxylate and quaternary ammonium portions of the molecules. Finally, a hypothesis is presented for the mode of binding of carnitine (or acetylcarnitine) to the enzyme, carnitine acetyltransferase. Based on this model for binding, a speculative topographic description of the enzymatic mechanism is presented. 

## INTRODUCTION

(*R*)-Carnitine is a naturally occurring compound found in both plant and animal tissues (*1*). High concentrations are located in vertebrates' heart and muscle tissues (*2*), which depend heavily upon fatty acid oxidation as an energy source. Carnitine is a substrate of the enzyme, carnitine acetyltransferase (EC 2.3.1.7), that catalyzes the reversible transfer of acetyl groups between carnitine and acetyl coenzyme A (*3*). Acylated carnitine acts as a carrier of fatty acyl groups across mitochondrial membranes in a vectorial transport mediated by a translocase enzyme (*4*). Carnitine has proven useful in the treatment of myocardial ischemia in animals. It facilitates the transfer of fatty acids in damaged areas, hence increasing energy production and promoting survivability of the tissue (*5*). In view of its central role in fatty acid oxidation, enhancing the knowledge of carnitine and its acyl transfer reactions is crucial for the development of its therapeutic potential. A knowledge of structures and conformational energies of both carnitine and acetylcarnitine is essential to an understanding of their mechanisms of action and their modes of binding to the enzyme.

Some methods of structural and conformational analyses have been applied to carnitine. These include MO calculations for gas-phase structures, NMR for solution conformation, and X-ray diffraction for determining solid-state structures. An

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electrostatic attraction involving the quaternary nitrogen and carboxylate anion of carnitine has been proposed (*6*). CNDO/2 calculations have supported (*7*) a possible role of electrostatics in favoring one conformation. Recent $^1$H NMR studies (*8, 9*) have identified the most populated conformations of carnitine and acetylcarnitine, and these conformations are similar to those seen in their crystal structures. The crystal structures of the hydrochloride salts of both carnitine and acetyl carnitine have been determined previously (*10–12*).

Since the extent of this electrostatic effect cannot be ascertained from analyses of the structures of the hydrochloride salts alone, single-crystal X-ray analyses have been conducted on the neutral zwitterions of both carnitine and acetylcarnitine. These zwitterionic species more accurately typify the charge state of these substrates because carboxy groups are expected to be fully ionized under physiological conditions. The structures of these zwitterions form the basis of this report. In addition, a hypothesis is presented for the mode of binding of carnitine (or acetylcarnitine) to carnitine acetyltransferase. Based on this model for binding, a speculative topographic description of the enzymatic mechanism is given.

## EXPERIMENTAL PROCEDURES

### *Materials*

Crystals of (*R*)-carnitine zwitterion were prepared by vapor diffusion of diethyl ether into a saturated solution of carnitine (Sigma Tau) in absolute methanol. The presence of water was strictly avoided during handling and preparation of the crystals.

(±)-Acetylcarnitine chloride was prepared from racemic carnitine (Aldrich) by "method A" of Ziegler *et al.* (*13*). The zwitterion was prepared by treatment with silver oxide in water at room temperature. In this way hydrolysis reactions observed with ion exchange resins were avoided. (±)-Acetylcarnitine zwitterion was crystallized by vapor diffusion of acetone into an isopropyl alcohol solution saturated with the zwitterion. (±)-Acetylcarnitine zwitterion was found to crystallize with one equivalent of water.

### *Diffraction Data*

Intensity data for each compound were collected on an Enraf–Nonius CAD4 diffractometer equipped with a graphite monochromator, using crystals sealed in thin-walled glass capillaries for protection from humidity. Data were measured at 25 ± 2°C by $\omega$-$2\theta$ scans of variable speeds, designed to yield $I \simeq 50\sigma(I)$ for all significant reflections. One quadrant of data was measured for each crystal.

Experimental details and unit cell information are listed in Table 1.

Data reduction included corrections for background, Lorentz, polarization and, in the case of (*R*)-carnitine, absorption effects. The absorption correction was based on $\Psi$ scans of reflections near $\chi = 90°$, and the minimum relative transmission coefficient was 0.9268.

TABLE 1

CRYSTAL DATA FOR CARNITINE AND ACETYLCARNITINE · $H_2O$

| | Carnitine | Acetylcarnitine · $H_2O$ |
|---|---|---|
| Formula | $C_7H_{15}NO_3$ | $C_9H_{17}NO_4 \cdot H_2O$ |
| Formula weight | 161.2 | 221.3 |
| Crystal system | Monoclinic | Monoclinic |
| Space group | $P2_1$ | $P2_1/c$ |
| $a$ (Å) | 7.342(1) | 12.315(3) |
| $b$ (Å) | 6.089(1) | 8.851(2) |
| $c$ (Å) | 9.530(2) | 11.109(3) |
| $\beta$ (deg.) | 97.58(1) | 95.02(2) |
| $V$ (Å$^3$) | 422.4(2) | 1206.2(8) |
| $Z$ (formulas/cell) | 2 | 4 |
| Radiation | CuK$\alpha$ | MoK$\alpha$ |
| $\lambda$ (Å) | 1.54184 | 0.71073 |
| $D_c$ (g cm$^{-3}$) | 1.267 | 1.218 |
| $\mu$ (cm$^{-1}$) | 8.25 | 0.92 |
| Crystal size (mm$^3$) | 0.16 × 0.36 × 0.60 | 0.36 × 0.40 × 0.56 |
| $\theta$ limits (deg.) | 2–75 | 1–25 |
| Unique data | 889 | 2119 |
| Observed data | 851 | 1136 |
| Variables | 160 | 198 |
| $R$ | 0.034 | 0.063 |
| $R_w$ | 0.049 | 0.084 |
| GOF | 1.95 | 2.75 |
| Extinction coefficient | 3.0(3) × 10$^{-5}$ | — |
| Maximum residual (eÅ$^{-3}$) | 0.17 | 0.40 |

*Structure Solution and Refinement*

*A. (R)-Carnitine.* The structure was solved by direct methods, MULTAN 78 (*14*), and refined by full-matrix least-squares based on $F$, with weights $\sigma(F_0)^{-2}$, using data for which $I > 3\sigma(I)$. Nonhydrogen atoms were treated anisotropically, while hydrogen atoms were located from difference maps and refined isotropically. The crystal suffered from slight secondary extinction, and an extinction parameter was refined.

*B. Acetylcarnitine monohydrate.* The structure elucidation proceeded virtually identically to that for (*R*)-carnitine. No extinction problem was encountered; however, a slight disorder involving the acetyl group was encountered. The molecule exists in the crystal in two equally populated conformations, differing by a rotation of approximately 40° about the O3—C8 bond. This disorder was represented in the refinement model by two half-populated positions for the carbonyl oxygen atom, O4, and also for the methyl group, C9. These half-atoms were refined isotropically, and the methyl hydrogen atoms were not located.

Coordinates for (*R*)-carnitine are given in Table 2; those for acetylcarnitine · $H_2O$ are given in Table 3; and anisotropic thermal parameters and structure factors are available from the authors.

TABLE 2

COORDINATES FOR (*R*)-CARNITINE

| Atom | *x* | *y* | *z* | Atom | *x* | *y* | *z* |
|---|---|---|---|---|---|---|---|
| O1 | 1.0079(1) | 0.7499(3) | 0.8039(1) | H3 | 0.930(2) | 0.759(4) | 1.071(1) |
| O2 | 0.7455(1) | 0.8512(4) | 0.6824(1) | H4 | 0.736(2) | 0.485(6) | 1.157(2) |
| O3 | 0.7408(1) | 1[a] | 1.0450(1) | H5 | 0.569(2) | 0.650(4) | 1.124(2) |
| N | 0.7340(1) | 0.7050(3) | 1.3083(1) | H6 | 0.629(3) | 0.918(8) | 1.445(2) |
| C1 | 0.8353(2) | 0.7681(4) | 0.7877(1) | H7 | 0.650(3) | 1.009(12) | 1.278(4) |
| C2 | 0.7328(2) | 0.6757(3) | 0.9034(1) | H8 | 0.489(3) | 0.841(7) | 1.312(3) |
| C3 | 0.7929(2) | 0.7769(3) | 1.0488(1) | H9 | 0.689(4) | 0.554(8) | 1.496(3) |
| C4 | 0.6980(2) | 0.6410(3) | 1.1529(1) | H10 | 0.560(3) | 0.481(7) | 1.356(3) |
| C5 | 0.6177(2) | 0.8953(5) | 1.3383(2) | H11 | 0.770(3) | 0.395(8) | 1.387(3) |
| C6 | 0.6845(2) | 0.5108(5) | 1.3920(2) | H12 | 0.969(2) | 0.877(5) | 1.311(2) |
| C7 | 0.9318(2) | 0.7561(4) | 1.3557(1) | H13 | 1.004(3) | 0.637(9) | 1.318(3) |
| H1 | 0.616(2) | 0.694(5) | 0.880(2) | H14 | 0.939(2) | 0.775(6) | 1.458(3) |
| H2 | 0.764(2) | 0.512(6) | 0.915(2) | H15 | 0.826(3) | 1.077(7) | 1.100(3) |

[a] The *y* coordinate of O3 was fixed to define the origin of the polar space group.

## RESULTS AND DISCUSSION

### *Crystal Structures*

Figure 1 shows the structures obtained for the zwitterions of carnitine and acetylcarnitine. The most distinct difference between the two structures is the

TABLE 3

COORDINATES FOR ACETYLCARNITINE MONOHYDRATE

| Atom | *x* | *y* | *z* | Atom | *x* | *y* | *z* |
|---|---|---|---|---|---|---|---|
| O1 | 0.1685(2) | 0.5691(3) | 0.5685(2) | H1 | 0.333(3) | 0.476(5) | 0.766(4) |
| O2 | 0.1869(3) | 0.3358(3) | 0.6352(3) | H2 | 0.217(3) | 0.505(4) | 0.819(3) |
| O3 | 0.3648(2) | 0.7269(3) | 0.8681(2) | H3 | 0.317(2) | 0.719(3) | 0.707(3) |
| O4[a] | 0.5028(6) | 0.7466(7) | 0.7509(7) | H4 | 0.169(3) | 0.787(4) | 0.864(3) |
| O4'[a] | 0.4986(5) | 0.6515(7) | 0.7632(6) | H5 | 0.122(3) | 0.747(3) | 0.745(3) |
| O5W | 0.9888(3) | 0.7582(3) | 0.5187(3) | H6 | 0.261(3) | 1.023(5) | 0.892(4) |
| N | 0.1878(2) | 0.9509(3) | 0.7381(2) | H7 | 0.330(4) | 1.010(5) | 0.778(4) |
| C1 | 0.2000(3) | 0.4741(4) | 0.6465(3) | H8 | 0.251(4) | 1.149(5) | 0.781(4) |
| C2 | 0.2599(3) | 0.5278(4) | 0.7626(3) | H9 | 0.068(4) | 1.006(5) | 0.850(4) |
| C3 | 0.2863(3) | 0.6952(4) | 0.7664(3) | H10 | 0.081(3) | 1.126(4) | 0.736(3) |
| C4 | 0.1867(3) | 0.7893(4) | 0.7807(3) | H11 | 0.016(4) | 0.955(6) | 0.686(5) |
| C5 | 0.2711(4) | 1.0424(4) | 0.8123(4) | H12 | 0.283(4) | 0.912(5) | 0.605(4) |
| C6 | 0.0769(3) | 1.0154(5) | 0.7529(4) | H13 | 0.156(5) | 0.890(7) | 0.550(5) |
| C7 | 0.2075(4) | 0.9621(4) | 0.6073(3) | H14 | 0.211(4) | 1.057(5) | 0.586(4) |
| C8 | 0.4697(4) | 0.7245(6) | 0.8522(4) | H1W | 0.935(3) | 0.723(4) | 0.481(3) |
| C9[a] | 0.5400(7) | 0.7260(11) | 0.9750(8) | H2W | 1.032(3) | 0.688(4) | 0.523(3) |
| C9'[a] | 0.5451(7) | 0.7882(11) | 0.9549(8) | | | | |

[a] Population = 1/2.

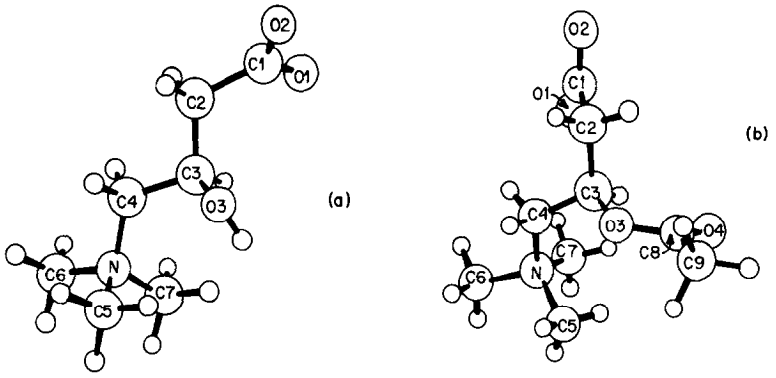



FIG. 1. (a) Solid-state conformation of carnitine zwitterion. (b) Solid-state conformation of acetylcarnitine zwitterion.

position of the carboxylate group. Carnitine exists in a fully "extended" conformation (C1—C2—C3—C4—N, *a-a*) whereas acetylcarnitine exists in a "folded" conformation (C1—C2—C3—C4—N, *g-a*) (*7*). A perceptible difference exists in the torsion angles C2—C3—C4—N and O3—C3—C4—N. The respective torsion angles are 156.4° and −83.1° for acetylcarnitine, but 179.7° and −61.5° for carnitine. Acetylation of the hydroxy group causes a rotation of approximately −22° about C3—C4.

Other significant differences are seen in the bond angles, C2—C3—C4 and O3—C3—C4. In carnitine, C2—C3—C4 is 6.1° smaller than in acetylcarnitine, 105.6° vs 111.7°. On the other hand, O3—C3—C4 is 5.3° larger, 113.1° vs 107.8°. Destro and Heyda (*12*) have suggested that this deformation is caused by the $N^+ \cdots O$ coulombic attraction. Acetylation of the hydroxy increases the O3—C3—C4—N torsion angle, and this is compensated for by compression of O3—C3—C4. There is a net increase in separation between the two groups as revealed by the O3—N nonbonded distance—3.09 Å, carnitine vs 3.20 Å, acetylcarnitine.

Another difference in the structures of carnitine and acetylcarnitine is in the relative orientation of the carboxylate groups to the backbone atoms. Since the oxygens are equivalent, the numbering, O1 or O2, is arbitrary. For carnitine (see Fig. 1a) C1—O2 is nearly eclipsing a C—H bond, while for acetylcarnitine (see Fig. 1b) C1—O1 is virtually eclipsing C2—C3. This may occur because of differences in hydrogen bonding. In carnitine, the hydroxyl group forms an intermolecular hydrogen bond with O1, having O---O separation of 2.663 Å and a 174° angle at H. The acetyl compound lacks hydrogen bond donors, and the water molecule serves this function. It donates hydrogen bonds to two acetylcarnitine molecules with parameters O5W---O2, 2.765 Å (angle at H = 169°) and O5W---O1, 2.792 Å (angle at H = 160°).

*Comparison with Previous Work*

A comparison of the zwitterionic structures with the hydrochloride salts (*10–12*) is made in Table 4. Selected bond distances, bond angles, torsion angles, and nonbonded distances are given. Aside from slight variations in torsion angles, the

TABLE 4

SELECTED BOND LENGTHS, BOND ANGLES, TORSION ANGLES, AND NONBONDED DISTANCES IN CRYSTAL STRUCTURES OF CARNITINE AND ACETYLCARNITINE

| Atoms | Carnitine | | Acetylcarnitine | | |
|---|---|---|---|---|---|
| | Zwitterion | Hydrochloride[a] | Monohydrate | Hydrochloride[b] | Hydrochloride · $H_2O$[c] |
| Bond lengths (Å) | | | | | |
| C1—O1 | 1.261(1) | 1.203(10) | 1.245(3) | 1.212(7) | 1.196(3) |
| C1—O2 | 1.234(2) | 1.324(10) | 1.239(3) | 1.328(7) | 1.313(3) |
| C1—C2 | 1.523(2) | 1.507(11) | 1.506(4) | 1.509(7) | 1.496(3) |
| C2—C3 | 1.527(1) | 1.529(11) | 1.516(4) | 1.525(7) | 1.518(3) |
| C3—O3 | 1.411(2) | 1.419(10) | 1.449(3) | 1.470(7) | 1.451(2) |
| C3—C4 | 1.528(2) | 1.519(11) | 1.503(4) | 1.517(7) | 1.504(3) |
| C4—N | 1.521(1) | 1.518(10) | 1.508(3) | 1.531(7) | 1.509(2) |
| Bond angles (deg.) | | | | | |
| O1—C1—O2 | 123.8(1) | 123.3(8) | 124.5(2) | 124.3(4) | 123.8(2) |
| O1—C1—C2 | 117.6(1) | 124.9(7) | 118.8(2) | 125.2(4) | 124.4(2) |
| O2—C1—C2 | 118.6(1) | 111.8(7) | 116.6(2) | 110.5(4) | 111.8(2) |
| C1—C2—C3 | 113.4(1) | 111.5(6) | 114.8(2) | 111.7(4) | 112.9(2) |
| C2—C3—C4 | 105.6(1) | 107.2(6) | 111.7(2) | 109.4(4) | 111.1(2) |
| C2—C3—O3 | 108.7(1) | 108.7(7) | 109.7(2) | 108.0(4) | 108.1(2) |
| O3—C3—C4 | 113.1(1) | 111.3(7) | 107.8(2) | 105.0(4) | 106.8(2) |
| C3—C4—N | 117.3(1) | 116.8(7) | 117.5(2) | 115.9(4) | 116.4(2) |
| Torsion angles (deg.) | | | | | |
| C1—C2—C3—C4 | −171.6(2) | −166.2 | −74.3(3) | −71.4 | −77.1 |
| C2—C3—C4—N | 179.7(2) | 174.8 | 156.4(3) | 156.3 | 158.9 |
| O1—C1—C2—C3 | 58.5(2) | 22.7 | 6.5(3) | −3.1 | 6.0 |
| C1—C2—C3—O3 | 66.7(2) | 73.4 | 116.3(3) | 174.9 | 166.0 |
| O3—C3—C4—N | −61.5(2) | −66.4 | −83.1(3) | −88.0 | −83.6 |
| C3—O3—C8—C9 | — | — | 167.5(7) [−147(7)][d] | −174.3 | −177.8 |
| Nonbonded distances (Å) | | | | | |
| C1—O3 | 3.02 | 2.99 | 3.78 | 3.78 | 3.74 |
| O3—N | 3.09 | 3.07 | 3.20 | 3.22 | 3.17 |
| C1—N | 5.13 | 5.06 | 4.35 | 4.24 | 4.33 |
| O1—N | 5.46 | 5.27 | 3.86 | 3.88 | 3.98 |

[a] Ref. (*10*).
[b] Ref. (*11*).
[c] Ref. (*12*).
[d] Two values, disorder in crystal.

overall conformations of the zwitterions closely match those of the hydrochlorides.

The data reveal no significant differences in bond distances except for protonated versus unprotonated carboxylates. The C1—O1 and C1—O2 distances for the zwitterions are nearly identical which is indicative of the resonant form.

Differences in the O1—C1—C2 versus O2—C1—C2 bond angles are seen for the hydrochlorides but not the zwitterions. The other angles involving the backbone atoms of the zwitterions and hydrochlorides are nearly the same. In all cases the C3—C4—N angle is unusually large because of steric repulsion between the trimethylammonium group and O3.

An examination of most of the torsion angles reveals close agreement among the zwitterionic and hydrochloride structures. A difference in the orientation of the carboxyl(ato) group (involving a twist about C1—C2) in carnitine is seen

between the hydrochloride and zwitterion. The data show that the O1—C1—C2—C3 torsion angle of the carnitine crystals differs by 36°. This shift in position appears to be due to differences in intermolecular H-bonding. This occurs between O2 and a chlorine atom of carnitine · HCl, whereas for the zwitterion an H-bond is formed between O1 and the hydroxyl group.

A unique difference in the position of the acetyl group of acetylcarnitine is observed for the zwitterion compared with the hydrochlorides. As previously noted, the acetyl group of acetylcarnitine · $H_2O$ exists in two distinct conformations related by rotation about the O3—C8 bond. This crystal disorder has not been reported with the hydrochloride salts.

Contributions from crystal packing forces cannot be excluded. However, both zwitterion and hydrochloride have the same backbone conformation. Since the same backbone conformation is observed in different crystals, the suggestion that this backbone conformation is independent of these forces is supported.

*Intramolecular Electrostatic Attraction Question*

It has been proposed (*6*) that the "folded" conformation is stabilized by an intramolecular ionic attraction between the carboxylate and the trimethylammonium group. This proposal has received additional support from EHT and CNDO/2 calculations (*7*) that have suggested that the low-energy conformation is stabilized by internal ionic forces.

This electrostatic interaction should be enhanced in the zwitterionic structures when compared to the hydrochlorides since carboxylate has more electron density on the oxygens than carbonyl. An examination of nonbonded distances O1—N and C1—N should give a measure of the attraction of the carboxylate to the ammonium group.

In carnitine, both the zwitterion and the hydrochloride have the same conformation, i.e., "extended." This means that the electrostatic attraction is not strong enough to favor the "folded" over the "extended." The factors which favor the "extended" conformation dominate. Intramolecular hydrogen bonding between the carboxylate and hydroxyl would stabilize the "extended" conformation. As seen in Fig. 1a, no such intramolecular hydrogen bond exists in the solid state. Therefore, there is no special effect that precludes formation of the "folded" conformation. If electrostatic attraction is a real effect, it is not large enough to overcome the factors that favor the "extended" conformation.

In acetylcarnitine, where both the zwitterion and the two hydrochlorides exist in the "folded" conformation, there is no significant shortening of the distance between the carboxylate and ammonium groups. The steric bulk of the acetyl group compared with hydroxyl biases acetylcarnitine toward the "folded" conformation. The proposed electrostatic attraction should result in further stabilization. Although the O1—N distance is the shortest in the zwitterion, the C1—N distance is the longest. Both distances are dependent on a number of conformational factors. If electrostatic attraction is a dominant factor, a more apparent shortening should be observed.

The conclusion reached at this point is that intramolecular electrostatic attrac-

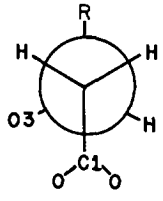
tion between carboxylate and the trimethylammonium group is a small effect or unimportant for determining the conformation of carnitine and acetylcarnitine in the solid state.

*Explanation of Conformational Preference*

The *gauche* conformation of the O3—C3—C4—N torsion angle in both structures has ample precedence. This is another example of the "*gauche* effect," i.e., the tendency for vicinal polar bonds to adopt a *gauche* conformation (*15*). Explanations, couched in the language of MO theory, of the *gauche* effect abound (*15, 16*). The preference for the *gauche* conformation in acetylcholine and its analogs (*17*) has been ascribed to be due in part to "conjugative destabilization" of the *anti* rotamer (*16b*). These MO descriptions complement previous suggestions for this preference based on electrostatic attraction (*18*) and dipole–dipole interaction (*19*).

An explanation for the difference in the conformation of the C1—C2—C3—C4 torsion angle, *gauche* in acetylcarnitine versus *anti* in carnitine, most likely resides in steric effects.

The *anti* conformation of the C1—C2—C3—C4 torsion angle in carnitine also has precedence. A search of the Cambridge Crystallographic Data Files (*20*) for compounds of the general formula, $RCH(OH)CH_2COOH$, has revealed that all 17 compounds[2] have the conformation shown below. Furthermore, this conforma-




tional preference holds for a series of hydrogen malates regardless of the nature of the cation (*21*). This conformational preference is a general feature of compounds of this class. The *gauche* relationship between hydroxy and carboxyl likely arises from the *gauche* effect and not intramolecular hydrogen bonding.

The *gauche* conformation of the C1—C2—C3—C4 torsion angle in acetylcarnitine does not have much precedence. A search of the Cambridge Crystallographic Data Files for the fragment, $C—OCH(R)CH_2COOH$, has uncovered only two structures besides acetylcarnitine. In both these structures, C3 and C4 are part of a five-membered ring which includes the oxygen attached to C3. The conformation in both is with C1—C2—C3—C4, *anti*, and C1—C2—C3—O, *gauche*. As discussed above, this is what is observed for 3-hydroxypropanoic acids.

This observed change in conformation about C2—C3 can be readily explained by steric effects. Acetylation of the hydroxyl in carnitine creates steric repulsion in the "extended" conformation, both with the trimethylammonium group and the

[2] Six different backbones: eight malic derivatives, five 3-aryl-3-hydroxypropanoic acids. Contact the authors for details of the search.

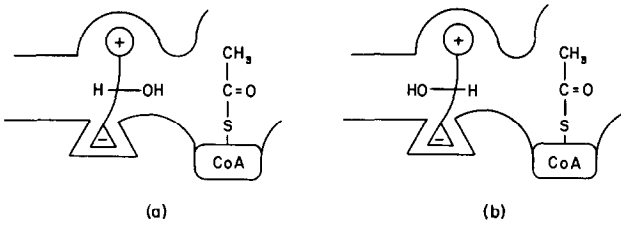



FIG. 2. Schematic of carnitine binding site on carnitine acetyltransferase. Carnitine makes a two-point attachment to the enzyme. The location of the CoASH binding site creates the third locus, thus creating the stereospecificity observed in the reaction. (a) (*R*) enantiomer is bound and acetyl transfer can occur. (b) (*S*) enantiomer is bound and acetyl transfer is not possible.

carboxylate group. This potential crowding between acetoxy and carboxylate is relieved by rotation to the "folded" conformation. The crowding between acetoxy and trimethylammonium is relieved by a rotation about C3—C4 of approximately −22°. The forces for preserving the *gauche* conformation for N—C4—C3—O are stronger than those for O—C3—C2—Cl.

In the related molecules, 4-aminobutanoic acid (GABA) and 4-amino-3-hydroxybutanoic acid (GABOB), the opposite occurs. GABA exists in the "folded" conformation in its crystal structure (*22*) while GABOB is in the "extended" (*23*). Craven and Weber (*22*) have proposed an intramolecular hydrogen bridging interaction in GABA as an explanation for the stability of the "folded" conformation. For GABOB in the "extended" conformation, the hydroxy group is *gauche* to the ammonium group and to the carboxy group. The structure of GABOB suggests that the *gauche* effect may be more important than intramolecular hydrogen bridging.

*Hypothesis for Conformer Selective Binding in Carnitine Acetyltransferase*

Carnitine acetyltransferase catalyzes the reversible transfer of an acetyl group between acetyl coenzyme A and (*R*)-carnitine (*3*). The enzyme is competitively inhibited by (*S*)-acetylcarnitine and (*S*)-carnitine (*24*).

Given this stereospecificity, a likely suggestion is that carnitine makes a two-point attachment to the enzyme (Fig. 2). The carboxylate and the trimethylammonium group should be the parts of the carnitine framework being recognized. Once anchored to the enzyme, the acetyl group can only be transferred when it is in proximity to the coenzyme A thiol group or the thiol group on the enzyme.[3] In order to allow for maximum steric accessibility about the hydroxyl group on carnitine, the enzyme should bind the "folded" conformation.

Murray *et al.* (*7*) have previously suggested that the conformations of carnitine and acylcarnitine play important roles in the activities of these various enzymes. They suggest that carnitine acetyltransferase requires the "extended" conformation of carnitine for the forward reaction. They adopt the carboxy participation mechanism, Fig. 3a, proposed by Fritz *et al.* (*3*), and explain how the "extended"

[3] A thiol group on the enzyme is needed for activity (*25*). Whether or not an acylenzyme is involved in the mechanism is not known.

conformation is needed for intramolecular transfer of the acetyl from carboxy to hydroxy. Once acetylated, they suggest the carboxy is free to rotate to the favored "folded" conformation. The only part of the molecule which remains firmly anchored to the enzyme is the quaternary ammonium group.

For the transfer of acetyl groups to and from carnitine and a thiol group, the following questions arise: Does the carboxyl group on carnitine participate? If not, then what is the most likely mechanism?

The carboxylato group probably does not participate. Studies (*26*) on model systems of intramolecular catalysis of acyl transfer by carboxylate would not support the formation of an anhydride intermediate. Since carnitine acetyltransferase catalyzes the reaction in both directions, the mechanism for the reaction should be reversible. The difficulty with mechanism (a) in Fig. 3 is that alkoxide is a much poorer leaving group than carboxylate. This disfavors formation of the high-energy anhydride. Furthermore, the carboxylate is needed for binding to the enzyme surface and would not be available for assisting the acetyl transfer.

A mechanism for *O* to *S* acyl transfer has been developed (*27*) in model reactions and is given in Fig. 3b. The reaction is simply an addition–elimination involving formation of a tetrahedral intermediate. Factors which affect the partitioning of this intermediate control the direction of the reaction.

Assuming the addition–elimination mechanism for the reaction, the question of which conformation of the carnitines is bound can be addressed. Close contacts

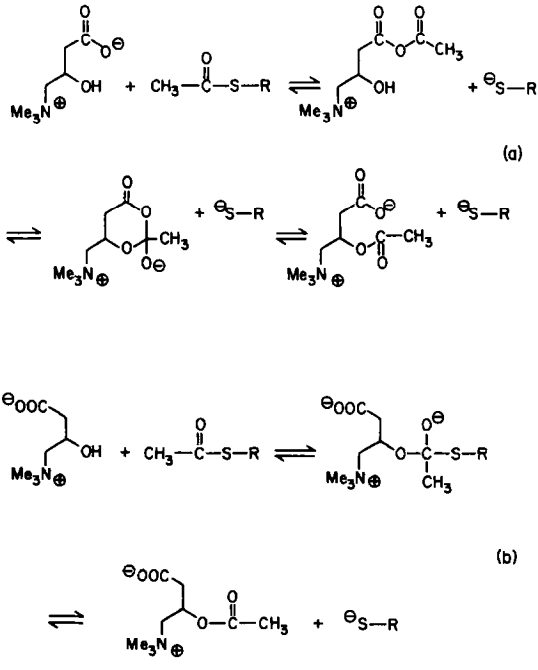



FIG. 3. (a) Mechanism of acetyl transfer proposed by Fritz *et al.* (*3*). (b) Typical mechanism for *O*- to *S*-acyl transfer, proton transfer steps not included.

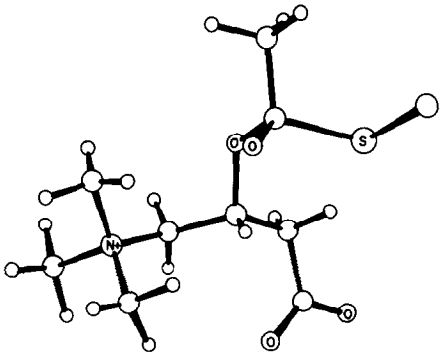



FIG. 4. Proposed conformation of tetrahedral intermediate in acetyl transfer between *O* and *S*. For steric and electronic reasons, *S* is placed opposite the trimethylammonium group.

between the carboxylate and the acetyl group disfavor the "extended" conformation and the "folded" conformation is observed in the crystalline state. Figure 4 shows the proposed conformation of enzyme-bound acetylcarnitine with the tetrahedral intermediate formed. These contacts are even closer when examining the tetrahedral intermediate. The "folded" conformation is most likely the one bound by the enzyme. The stereospecificity of the reaction and the lack of necessity for carboxylate participation argue for a firm attachment of the carboxylate to its binding site.

Figure 4 further suggests a topographic arrangement in the enzyme with respect to the position of the thiol group. Assuming the conformation of the acetoxy group in the crystalline state is similar to that in the enzyme–substrate complex, the thiol most likely will attach to the *Si* face of the acetoxy carbonyl from the carboxy side of carnitine. The trimethylammonium group is too bulky to allow attack from its side. Furthermore, development of negative charge on oxygen as the acetoxy group is attacked, will be stabilized by the quaternary ammonium group.

In conclusion, a hypothesis has been presented which describes the topographical and conformational arrangement of molecules involved in the transfer of acetyl groups to and from carnitine. This hypothesis is consistent with current information concerning the enzymatic reaction as well as its bioorganic chemistry.

Work is in progress in these laboratories on the preparation of analogs to test this hypothesis.

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