Conformationally-Dependent Free Energies of Solvation. An Explanation for the Large Group-Transfer Potential of Acetylcarnitine

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Carnitine (Car) and Car acyltransferases are crucial in regulating the rates at which long-chain fatty acids are oxidized in the mitochondria of mammalian tissue.[1] As a specific cofactor, Car transports fatty acyl groups across the inner mitochondrial membrane via a Car−acylCar translocase.[2] Car acyltransferases mediate transfer of acyl groups from cytosolic coenzyme A (CoA) to Car in the outer mitochondrial membrane and from Car to mitochondrial CoA on the inside of the inner membrane. Because acyl-CoA, a thioester, has a large group-transfer potential, acylCar, an oxyester, must have a similar potential to avoid coupling the transfer reaction to an energy-releasing reaction. To illustrate, $\Delta G°_{hyd}$ equals −8.2 and −7.9 kcal/mol for acetyl-CoA[3] and acetylcarnitine (AcCar),[4] respectively. Oxyesters typically have 2.3 kcal/mol lower group-transfer potentials than thioesters.[5] Acetylcholine (AcCh), which is structurally related to AcCar, has a $\Delta G°_{hyd}$ of −6.47 kcal/mol.[5] Why acylCar[6] has a larger group-transfer potential than AcCh has remained a mystery for over three decades.[4]

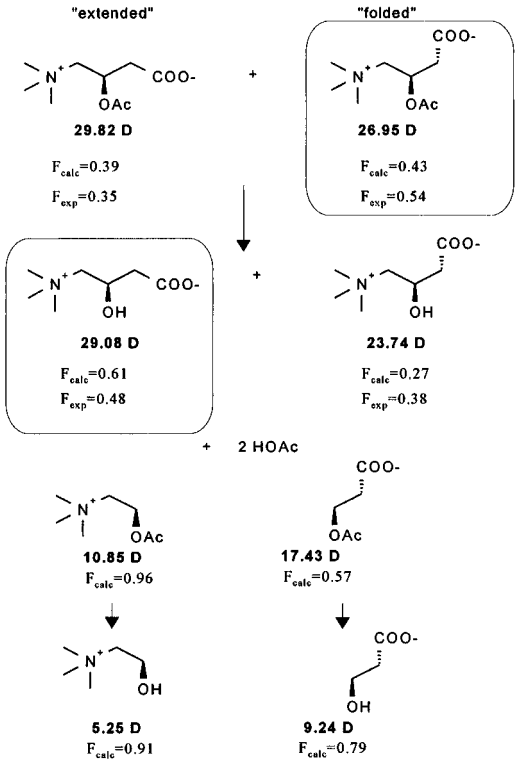
Solvation energies can determine the reactivity of "high-energy" compounds.[7] For highly charged molecules, greater solvation energies of products than those of reactants may overcome decreases in bond strengths. In high-energy phosphates, such as ATP,[8] the large solvation energy of the dianionic product, phosphate, drives the reaction. Strong solvation of the products substantially increases the $\Delta G°_{hyd}$ for a series of uncharged phosphoric and carboxylic anhydrides.[9] Ring−chain tautomerism in sugars behaves similarly because the solvation energy of the anomeric hydroxyl group is anomalously high.[10]

With more powerful computers, computational chemists strive to develop calculational methods to enable the correct inclusion of solvent effects.[11] We can now unravel the influence of solvation on the thermodynamics of biological molecules.

We report herein semiempirical computations, including solvation, that reproduce qualitatively the conformational populations of AcCar, Car,[12] AcCh, and choline (Ch).[13] Our results suggest that the hydrolysis of AcCar owes the large $\Delta G°_{hyd}$ to a change in the conformer populations in going from AcCar to Car. This change in conformational populations explains the relatively large solvation energy of Car.

Our earlier conformational analyses[12] of AcCar and Car revealed that the preferred conformer of AcCar labeled "folded"[14] (Scheme 1), becomes less populated after hydrolysis and that the preferred conformation of Car is "extended". To investigate

Scheme 1



the effect of this population shift on solvation, we used AM1 and the COSMO[11i] solvent model, because AM1 allows a complete conformational search with the quality of atomic charges needed for COSMO. We performed a few ab initio calculations to check for consistency with the AM1 results.[15]

A complete study by ab initio methods was impractical. For a calibration check, we compared the energies of selected pairs of minima in gas phase, fully optimized by AM1 vs fully optimized by HF/6-31G* and single-point by MP2 on fully optimized HF/6-31G*. We found (Table 1) small differences in the relative energies. The AM1-optimized geometries were used as input for HF/6-31G*. The results for AcCh and Ch in the gas phase followed the same trends in AM1 and ab initio

(1) *Current Concepts in Carnitine Research*; Carter, A. L., Ed.; CRC Press: Boca Raton, FL, 1992.
(2) Pande, S. V. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 883−887.
(3) Burton, K. *Biochem. J.* **1955**, *59*, 44−46. Burton, R. M.; Stadtman, E. R. *J. Biol. Chem.* **1953**, *202*, 873−890.
(4) Fritz, I. B.; Schultz, S. K.; Srere, P. A. *J. Biol. Chem.* **1963**, *238*, 2509−2517.
(5) Jencks, W. P.; Cordes, S.; Carriuolo, J. *J. Biol. Chem.* **1960**, *235*, 3608−3614.
(6) Microcalorimetry suggests that this value changes little with longer acyl chains. Müller, D. M.; Strack, E. *Hoppe-Seyler's Z. Physiol. Chem.* **1973**, *354*, 1091−1096.
(7) George, P.; Witonsky, R. J.; Trachtman, M.; Wu, C.; Dorwart, W.; Richman, L.; Richman, W.; Shurayh, F.; Lentz, B. *Biochim. Biophys. Acta* **1970**, *223*, 1−15.
(8) Hayes, D. M.; Kenyon, G. L.; Kollman, P. A. *J. Am. Chem. Soc.* **1978**, *100*, 4331−4340.
(9) Wolfenden, R.; Liang, Y.-L. *Bioorg. Chem.* **1989**, *17*, 486−489.
(10) Wolfenden, R.; Liang, Y.-L. *J. Biol. Chem.* **1988**, *263*, 8022−8026.
(11) (a) Onsager, L *J. Am. Chem. Soc.* **1936**, *58*, 1486−1493. (b) Rivail, J.-L.; Rinaldi, D. *Chem. Phys.* **1976**, *18*, 233−242. (c) Warshel, A.; Levitt, M. *J. Mol. Biol.* **1976**, *103*, 227−249. (d) Tapia, O. In *Molecular Interactions*; Ratajczak, H., Orville-Thomas, W. J., Eds.; John Wiley & Sons: Chichester, 1982; Vol. 3, pp 47−118. (e) Ooi, T.; Oobatake, M.; Némethy, G.; Scheraga, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3086−3090. (f) Aguilar, M. A.; Olivares del Valle, F. J. *Chem. Phys.* **1989**, *129*, 439−450. (g) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129. (h) Cramer, C. J.; Truhlar, D. G. *Science* **1992**, *256*, 213−216. (i) Klamt, A.; Schüürman, G. *J. Chem. Soc., Perkin Trans. 2* **1993**, 799−805. (j) Cramer, C. J.; Truhlar, D. G. In *Quantitative Treatments of Solute/Solvent Interactions*; Politzer, P., Murray, J. S., Eds.; Elsevier: Amsterdam, 1994; Chapter 1, pp 9−54. (k) Cramer, C. J.; Truhlar, D. G. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; VCH Publishers, Inc.: NewYork, 1995; Vol. 6, pp 1−72.
(12) Colucci, W. J.; Gandour, R. D.; Mooberry, E. A. *J. Am. Chem. Soc.* **1986**, *108*, 7141−7147.
(13) (a) Partington, P.; Feeney, J.; Burgen, A. S. V. *Mol. Pharmacol.* **1972**, *8*, 269−277. (b) Lichtenberg, D.; Kroon, P. A.; Chan, S. I. *J. Am. Chem. Soc.* **1974**, *96*, 5934−5936.
(14) Murray, W. J.; Reed, K. W.; Roche, E. B. *J. Theor. Biol.* **1980**, *82*, 559−572. Reed, K. W.; Murray, W. J.; Roche, E. B. *J. Pharm. Sci.* **1980**, *69*, 1065−1068.
(15) Gaussian 94 (Revision C.2); Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. A.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1995.

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Table 1. Comparison of AM1 vs 6-31G* in the Gas Phase

	relative energies			μ (D)	
compound[a]	AM1	6-31G*	MP2	AM1	6-31G*
acetylcarnitine	0.00	0.00	0.00	12.15	10.72
	0.20	−0.66	0.86	12.66	12.81
carnitine	0.00	0.00	0.00	12.74	12.22
	0.94	−0.86	−0.50	13.42	12.54
acetylcholine	0.00	0.00	0.00	5.92	6.16
	0.45	0.55	1.83	6.20	6.95
choline	0.00	0.00	0.00	2.34	1.86
	4.89	4.46	5.76	4.10	3.64

[a] The first entry for each compound is the global minimum. The second entry is the second most populated conformer by AM1.

Table 2. Comparison of Dipole Moments of Global Minima in Water by AM1/COSMO vs 6-31G*/Tomasi

	μ	
compound	COSMO	Tomasi
acetylcarnitine	26.67	26.67
carnitine	29.17	28.87
acetylcholine	10.85	10.37
choline	5.25	5.03

for the energies as well as the dipoles. Inclusion of correlation (single-points MP2//HF/6-31G* on optimized structures) switched the results for AcCar (Table 1).[16]

Another concern was could COSMO reproduce the change in the dipole moment when adding a solvent model? We used the global minima from AM1/COSMO as input for single-point calculations using the Tomasi solvent model at the HF/6-31G* level (Table 2). Full optimization at the HF/6-31G*(Tomasi) level proved impractical. The Tomasi method calculates μ values similar to those obtained by COSMO.

From these comparisons, we deduced that the final conclusions of a full conformational search by ab initio methods would not differ qualitatively with those produced by AM1.

Computations using AM1 and AM1/COSMO as implemented in MOPAC 6.0[17] and MOPAC93, respectively, reproduced qualitatively the Boltzmann factors (F_{exptl})[12] for conformations of AcCar and Car. For reference, we also calculated the conformational populations of AcCar, Ch, 3-acetoxypropanoate, and 3-hydroxypropanoate. We performed a full grid search to locate the global minima. All conformers up to 3.5 kcal/mol above the global minimum were used[18] to calculate Boltzmann factors F_{calcd} for the compounds in gas phase and in solution. We used these values of F_{calcd} to predict expectation values for ΔH_f and μ. We used ΔH, and not ΔG, because the measured[6] difference in ΔS between the hydrolyses of AcCar and AcCh is <1 eu.[19]

The computations magnify the trend in ΔH_{hyd} (Table 3) when comparing AcCar and AcCh, i.e., $\Delta\Delta H_{\text{hyd}}$ (computed) = 4.37 kcal/mol compared to $\Delta\Delta H_{\text{hyd}}$ (experimental) = 1.67 kcal/mol. The difference among the three ΔH_{hyd} values resides in the $\Delta\Delta H_{\text{solv}}$ between the ester and the alcohol: 2.36 (AcCar−Car), 4.43 (AcCh−Ch), and 4.48 kcal/mol (3-acetoxypropanoate−3-hydroxypropanoate).

The $\Delta H^\circ_{\text{solv}}$ decreases with $\langle\mu\rangle$ within a pair of molecules, ester and alcohol (Table 4 for solution-phase dipoles (point-charges); for the ions, the origin was the center of mass of the molecule). The $\langle\mu\rangle$ of (Ac)Car closely depends on the CH_2-$CH-CH_2COO^-$ torsion angle, which influences the distance between the charges in the molecule. The $NCH_2-CH(O)$ torsion angle can affect this distance, but in solution this torsion angle is "fixed"(>90% in g^- conformation for the (Ac)Car), due to the "gauche effect".[20,21] From calculations, more than 98% of the conformers have the $NCH_2-CH(O)$ torsion angle in g conformation (g for the (Ac)Ch and g^- for the (Ac)Car). Scheme 1 shows the marked change in μ in solution when changing the most populated conformation (circumscribed) from "folded" to "extended".

Table 3. Comparison of Calculated vs Experimental $\Delta H^\circ_{\text{hyd}}$ (kcal/mol)

	ΔH_{hyd}		
compound	calcd	no buffer	pH = 7.0
acetylcarnitine	−7.43	−4.63[a]	−14.67[a]
acetylcholine	−3.06	−3.20[a]	−13.00[a]
3-acetoxypropanoate	−4.02	nd[b]	nd[b]

[a] Reference 6. [b] nd: not determined.

Table 4. Dipole Moments in Solution and $\Delta H^\circ_{\text{solv}}$ Calculated by AM1/COSMO (Exptl Values Measured in Water)

compound	$\langle\mu\rangle$	$\Delta H^\circ_{\text{solv}}$ (kcal/mol)
acetylcarnitine	27.90	−64.76
carnitine	27.12	−62.35
acetylcholine	9.89	−59.30
choline	4.79 (2.65 exptl)[a]	−54.87
3-acetoxypropanoate	16.07	−94.54
3-hydroxypropanoate	8.93	−90.06
acetic acid	6.40	−17.02 (−12.7 exptl)[b]
water	2.22	−9.22 (−9.98 exptl)[c]

[a] Maurel, P.; Galzigna, L. *Biophys. J.* **1971**, *11*, 550−557. [b] Wilson, B.; Georgiadis, R.; Bartmess, J. E. *J. Am. Chem. Soc.* **1991**, *113*, 1762−1766. [c] BenNaim, A. *Solvation Thermodynamics*; Plenum Press: New York 1987; p 81.

The zwitterion in both AcCar and Car creates a high polarity in each. The calculations overestimate the $\langle\mu\rangle$ for both AcCar and Car; because, for both, calculations underestimate the population of "folded" (Scheme 1).

If the distance between charges control solvation, then neutralization of charge will eliminate "excess" chemical energy. The methyl ester of AcCar hydrolyzes with $\Delta H_{\text{hyd}} = -13.02$ kcal/mol (pH = 7.0), close to that of AcCh. Computed ΔH_{hyd} values of AcCh and 3-acetoxypropanoate (Table 3) suggest that neither has a large group-transfer potential.

Our results reproduce qualitatively the experimentally determined conformational populations of Car and AcCar,[12] as well as the trends for $\Delta H^\circ_{\text{hyd}}$ values of AcCar and AcCh.[6] The similar polarity of AcCar and Car, which arises from the conformational change, results in a smaller $\Delta\Delta H^\circ_{\text{solv}}$ than that calculated for AcCh and Ch. The smaller $\Delta\Delta H^\circ_{\text{solv}}$ of the carnitines compared to that of the cholines serves as a mechanism for the release of the chemical energy that gives acylCar a large group-transfer potential. This finding that conformationally-dependent solvation energies can increase group-transfer potentials not only resolves a 34-year old mystery, but suggests that other biological processes involving zwitterions may use a similar mechanism to drive chemical reactions.

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(16) We are currently working to determine the origin of the discrepancy between cations and zwitterions by ab initio calculations.

(17) Stewart, J. J. P.; MOPAC: a General Molecular Orbital Package. *Quant. Chem. Prog. Exch.* **1990**, *10*, 86.

(18) All calculations were made on a Silicon Graphics Indigo^2 Extreme workstation equipped with a R4400 CPU, 96 MB of RAM and IRIX 5.3. PCMODEL v.4.0 (Serena Software, Bloomington, IN) was used to generate *Z*-matrices for MOPAC 6.0 and MOPAC 93. The optimizations were carried on until GNORM < 0.01 using BFGS (in gas phase) or until GNORM < 0.3 using EF (in solution, EPS = 78.3).

(19) Our values in solution are not strictly enthalpies, given that the inclusion of a continuum solvent potential implies an entropic contribution in the solvation term (see ref 11d). We thank one of the reviewers for calling our attention to this point.

(20) Sundaralingam, M. *Nature (London)* **1968**, *217*, 35−37.

(21) Wiberg, K. B.; Murcko, M. A.; Laidig, K. E.; MacDougall, P. J. *J. Phys. Chem.* **1990**, *94*, 6956−6959.