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Thermodynamics of Fatty Acid Degradation

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In this communication, the correlation of some thermodynamic properties of structurally similar biological compounds with items such as the number of carbon atoms or the number of its characteristic groups, such as phosphates, was applied to enzyme thermodynamics, to advance the thermodynamics of fatty acid degradation. It was shown that these concepts apply equally well for all three major thermodynamic functions: Gibbs energy of formation, enthalpy of formation, and entropy of formation. First, the standard transformed Gibbs energies of formation, $\Delta_f G'^o$, were calculated for the major portion of saturated fatty acids at 25 °C and pH 5, pH 7, and pH 9. Second, a total ATP yield was calculated for complete combustion of major saturated fatty acids to CO_2 and water. Third, the standard transformed Gibbs energies of reaction, $\Delta_r G'^o$, were calculated for enzyme reactions catalyzing complete β-oxidation of butyryl-CoA to acetyl-CoA at 25 °C and pH 7.

Introduction

Half a century ago, Benson proposed a set of additivity rules for the estimation of thermodynamic properties of chemical compounds.[1] The experimental data covered in that communication have been later greatly expanded by Benson to several hundreds of chemical compounds and sorted out into a number of separate groups according to their chemical structures;[2,3] all data reported by Benson referred to compounds in the gas phase.

One of the most prevalent techniques for estimating the standard Gibbs free energy of formation ($\Delta_f G'^o$) for a wide variety of biological compounds is the group contribution method of Mavrovouniotis.[4−6] This method allows the rapid calculation of $\Delta_r G'^o$ and equilibrium constants of biological reactions. Unlike the group contribution method of Benson,[3] this method is tailored for aqueous organic reactions taking place at neutral pH involving ionic species.[4,5]

In this work, we have assumed that there are correlations of some thermodynamic properties of structurally similar biological compounds with items such as the number of carbon atoms or the number of its characteristic groups such as phosphates. When we applied these concepts to thermodynamic properties of some structurally similar biological compounds, we obtained the results shown in Figures 1 and 2.

Figure 1 shows the correlation between the number of carbon atoms in sugar phosphates, glyceraldehyde-3-phosphate, erythrose-4-phosphate, ribose-5-phosphate, glucose-6-phosphate, and sedoheptulose-7-phosphate, and the corresponding standard transformed Gibbs energies of formation. The intercept on the ordinate is −864.7 kJ/mol, which indicates the standard transformed Gibbs energy of formation for the ester-bound orthophosphate group. Figure 2 shows the correlation between the number of phosphate groups in AMP, ADP, and ATP and the corresponding standard transformed Gibbs energies of formation. The slope of the correlation is −876.9 kJ/mol, which again indicates the standard transformed Gibbs energy of formation for the bound orthophosphate group. Thus, both values

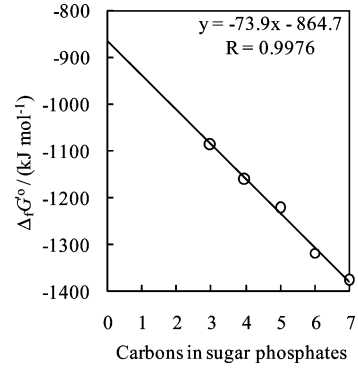

Figure 1. Correlation between the number of carbons in sugar phosphates and the standard transformed Gibbs energy of formation, $\Delta_f G'^o$, at pH 7, 25 °C.

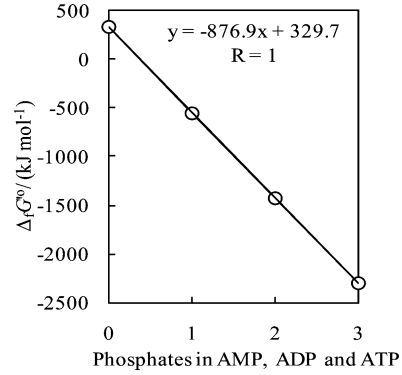

Figure 2. Correlation between the number of phosphates in AMP, ADP, and ATP and the standard transformed Gibbs energy of formation, $\Delta_f G'^o$, at pH 7, 25 °C.

(−864.7 and −876.9 kJ/mol) refer to the standard transformed Gibbs energy of formation for the bound orthophosphate group. The intercept on the ordinate in Figure 2 amounts to 329.7 kJ/mol, which indicates the standard transformed Gibbs energy of formation for a parent compound without orthophosphate groups, i.e., to adenosine. These correlations work also if values of $\Delta_f G^\circ$ for chemical species or $\Delta_f G'^o$ at other pHs are used.

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TABLE 1: Standard Transformed Gibbs Energies of Formation $\Delta_f G'^o$ in kJ/mol at 25°C and Ionic Strength 0.25 M[a]

no.	compound	pH 5	pH 7	pH 9	reference
1	acetic acid	−282.71	−247.83	−213.57	7
2	acetoacetyl-CoA	−138.57	−81.49	−24.41	7
3	acetoacetate	−335.74	−278.66	−221.58	7
4	acetyl-CoA	−92.31	−58.06	−23.81	7
5	adenosine		335.46		8
6	AMP		−554.87		8
7	ADP		−1425.96		8
8	ATP		−2297.34		8
9	butyric acid	−147.99	−68.08	11.83	7
10	butyryl-CoA	40.76	122	202	Thiswork
11	CoA	−18.48	−7.26	−1.10	7
12	CO_2(total)	−564.61	−547.10	−535.80	7
13	erythrose-4-phosphate	−1237.59	−1161.86	−1092.88	9
14	FAD_{ox}	906.61	1260.51	1614.40	7
15	FAD_{red}	876.71	1253.44	1630.17	7
16	formic acid	−322.46	−311.04	−299.63	7
17	glucose-6-phosphate	−1449.53	−1318.92	−1193.18	7
18	glyceraldehyde-3-phoshate	−1147.22	−1088.04	−1030.93	7
19	H_2O	−178.49	−155.66	−132.83	7
20	NAD_{ox}	762.29	1059.11	1355.92	7
21	NAD_{red}	811.86	1120.09	1428.33	7
22	orthophosphate	−1059.49	−1047.17	−1079.46	7
23	pyrophosphate	−1957.07	−1940.66	−1933.29	7
24	palmitic acid	649.64	1003.54	1357.43	7
25	ribose-5-phosphate	−1341.45	−1232.43	−1129.38	7
26	sedoheptulose-7-phosphate	−1531.48	−1379.04	−1230.53	9

[a] The values were taken from Alberty,[7] Boerio-Goates et al.,[8] and Leskovac et al.[9] or calculated in this study.

The above two examples indicate the power and accuracy of the additivity approach. This approach requires a set of closely related compounds, differing only by a single chemical group, and a good linear relationship between the number of carbon atoms or the number of the characteristic groups in the compounds and their respective standard transformed Gibbs energies of formation.

The additivity concepts of Benson[1] and Mavrovouniotis[4,5] have been applied in this communication for a specific purpose, to analyze the thermodynamic properties of enzyme-catalyzed reactions involving the degradation of lipids i.e., saturated fatty acids in living organisms; all data reported in this communication refer to the compounds in aqueous solution. To apply the additivity method to standard transformed Gibbs energies of formation, it is necessary to have reliable sources of data for biological compounds that are under investigation. Table 1 shows the standard transformed Gibbs energies of formation, $\Delta_f G'^o$, in kilojoules/mole, at ionic strength 0.25 M and 25 °C, at pH 5, pH 7, and pH 9, the conditions which appear to be close to physiological. The table displays only the values for compounds that are investigated in this communication.

Saturated Fatty Acids in Biology. Saturated fatty acids in living organisms arise from the hydrolysis of lipids, which are in most cases fatty acid esters with the alcohol glycerol. Free fatty acids are poisonous for the cell because they are uncouplers of oxidative phosphorylation in mitochondria.[10] Therefore, they are promptly esterified with CoA into the fatty acyl-CoA esters, once they are found inside the cell. Table 2 displays the list of important naturally occurring fatty acids.

Calculation of Standard Transformed Gibbs Energies of Formation for Saturated Fatty Acids. The first task in this section is to calculate the standard transformed Gibbs energy of formation, $\Delta_f G'^o$, for free saturated fatty acids listed in Table 2. There are four saturated carboxylic acids available

TABLE 2: Some Naturally Occurring Saturated Carboxylic Acids in Mammals[11]

carbons	compound	common name	IUPAC name
1	HCOOH	formic acid	methanoic acid
2	CH_3COOH	acetic acid	ethanoic acid
3	CH_3CH_2COOH	propionic acid	propanoic acid
4	$CH_3(CH_2)_2COOH$	butyric acid	butanoic acid
5	$CH_3(CH_2)_3COOH$	valeric acid	pentanoic acid
6	$CH_3(CH_2)_4COOH$	caproic acid	hexanoic acid
8	$CH_3(CH_2)_6COOH$	caprylic acid	octanoic acid
10	$CH_3(CH_2)_8COOH$	capric acid	decanoic acid
12	$CH_3(CH_2)_{10}COOH$	lauric acid	dodecanoic acid
14	$CH_3(CH_2)_{12}COOH$	myristic acid	tetradecanoic acid
16	$CH_3(CH_2)_{14}COOH$	palmitic acid	hexadecanoic acid
18	$CH_3(CH_2)_{16}COOH$	stearic acid	octadecanoic acid
20	$CH_3(CH_2)_{18}COOH$	arachidate	eicosanoate

from Table 1: formic, acetic, butyric, and palmitic acids. Figure 3 shows the correlation of the number of carbon atoms in the molecules of these fatty acids and the corresponding standard transformed Gibbs energies of their formation. Figure 3 shows a high degree of correlation between the number of carbon atoms and the corresponding Gibbs energies of formation at pH 7.

From the data in Table 1, it is possible to calculate the correlations between the standard transformed Gibbs energies of formation for formic, acetic, butyric, and palmitic acid, in the same way as in Figure 3, at pH 5, 7, and 9

$$\text{At pH 5: } \mathbf{y} = 65.697\mathbf{x} - 403.64 \quad (R = 0.9997) \tag{1}$$

$$\text{At pH 7: } \mathbf{y} = 88.513\mathbf{x} - 414.8 \quad (R = 0.9998) \tag{2}$$

$$\text{At pH 9: } \mathbf{y} = 111.34\mathbf{x} - 426.21 \quad (R = 0.9999) \tag{3}$$

Each of the above correlations was then expanded by varying the abscissa value (**x**) from 1 to 20, and so the standard transformed Gibbs energies of formation for 12 saturated fatty acids at pH 5, pH 7, and pH 9 were calculated (Table 3).

Degradation of Saturated Fatty Acids in Mitochondria. The biological degradation of fatty acids in mammals takes place by β-oxidation, which is a stepwise oxidative removal of two carbon units from the carboxyl end of the acid. The process takes place in mitochondria, and the fatty acids must be activated outside mitochondria before they enter the mitochondrial

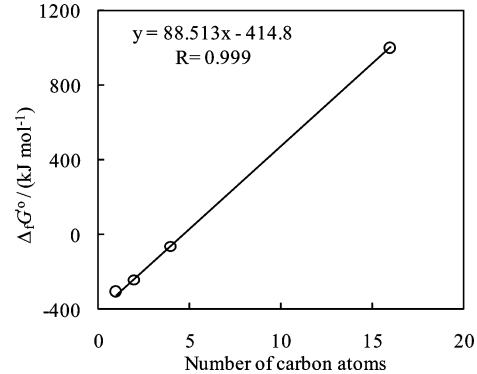

Figure 3. Correlation between the number of carbon atoms in formic, acetic, butyric, and palmitic acids, and their standard transformed Gibbs energy of formation, $\Delta_f G'^o$, at pH 7, 25 °C, and ionic strength 0.25M, plotted from the data in Table 1.

TABLE 3: Standard Transformed Gibbs Energies of Formation, $\Delta_f G'^{o}$, for Saturated Carboxylic Acids at 25°C, in kJ/mol

carbons	formula	pH 5	pH 7	pH 9
1	HCOOH	−337.94	−326.29	−314.87
2	CH_3COOH	−272.25	−237.77	−203.53
3	CH_3CH_2COOH	−206.55	−149.26	−92.19
4	$CH_3(CH_2)_2COOH$	−140.85	−60.75	19.15
6	$CH_3(CH_2)_4COOH$	−9.46	116.28	241.83
8	$CH_3(CH_2)_6COOH$	121.94	294.30	464.51
10	$CH_3(CH_2)_8COOH$	253.33	470.33	686.79
12	$CH_3(CH_2)_{10}COOH$	384.72	647.36	909.87
14	$CH_3(CH_2)_{12}COOH$	516.12	824.38	1132.55
16	$CH_3(CH_2)_{14}COOH$	647.51	1001.41	1355.23
18	$CH_3(CH_2)_{16}COOH$	778.91	1178.43	1577.19
20	$CH_3(CH_2)_{18}COOH$	910.30	1355.46	1800.59

matrix.[12−14] The activation reaction occurs on the outer mitochondrial membrane, catalyzed by acyl-CoA synthetases:

$$RCOOH + ATP + CoA \rightleftarrows RCO\text{-}CoA + AMP + PP_i \quad (4)$$

The enzyme acyl-CoA synthetases catalyze reaction 4 with short chain fatty acids (EC 6.2.1.2) or with long chain fatty acids (EC 6.2.1.3). For example, the reaction:

$$CH_3COOH + ATP + CoA \rightleftarrows CH_3CO\text{-}CoA + AMP + PP_i \quad (5)$$

is catalyzed by an enzyme acetyl-CoA synthetase (EC 6.2.1.1). The change in Gibbs energy $\Delta_r G'^{\circ}$, standard transformed Gibbs energy, for reaction 5 is −6.1 kJ/mol at pH 7 and 25 °C.[15] Thus, the activation of a fatty acid by formation of its ester with coenzyme A is the first step in their degradation. Initially, the coenzyme A ester of the fatty acid is oxidized by FAD_{ox} to produce an enoyl-CoA compound. The enoyl-CoA compound is hydrated, producing a corresponding hydroxyl compound. The hydroxyl group is then oxidized by NAD_{ox} into the corresponding keto group, and the ensuing carbonyl compound is split by coenzyme A into a fatty acyl-CoA ester, shorter by two carbon atoms, and acetyl-CoA.[10,12−14] Table 4 lists the enzyme reactions involved in degradation of principal saturated fatty acids.

The products of fatty acid oxidation are acetyl-CoA and reduced coenzymes, FAD_{red} and NAD_{red}. Each molecule of acetyl-CoA is oxidized in the citric acid cycle into two molecules of CO_2. The energy released by this oxidation is conserved in the reduction of three molecules of NAD^+ and one molecule of FAD and the production of one molecule of GTP.

The oxidative phosphorylation in mitochondria completes the oxidation of fatty acids by molecular oxygen by producing three ATP molecules from ADP and orthophosphate for each NAD_{red} molecule reoxidized into NAD_{ox} and by producing two ATP molecules from ADP and orthophosphate for each FAD_{red} molecule reoxidized into FAD_{ox}.[10,12−14] Combining the above information, it is easy to calculate the number of ATP molecules synthesized by the complete degradation of each fatty acyl-CoA compound in turn that is listed in Table 4. The net yield for the complete oxidation of each free fatty acid in Table 4 is in fact higher by two molecules of ATP, which are initially consumed for the activation of fatty acid into the corresponding fatty acyl-CoA ester (eq 5).

The yield of ATP in the complete combustion of fatty acids is remarkable. For each two additional methylene groups, an additional 17 ATP molecules are synthesized. This high yield for the complete oxidation of fatty acids to CO_2 and water reflects the high degree of their content in hydrogen atoms and by their very low content in oxygen atoms.

Degradation of Butyryl-CoA by β-Oxidation. Let us consider the degradation of the shortest acyl-CoA compound that is subject to β-oxidation, i.e., butyryl-CoA. Initially, the coenzyme A ester of the fatty acid is oxidized by FAD_{ox} to

TABLE 4: Stoichiometry of Coenzyme Reduction and ATP Formation in the Enzymatic Reactions for the Degradation of Saturated Fatty Acyl-CoA Esters by β-Oxidation in Mitochondria

carbons	reaction	ATP yield[a]
20	$CH_3(CH_2)_{18}COCoA + 9FAD_{ox} + 9NAD_{ox} + 9CoA + 9H_2O \rightarrow 10acetyl\text{-}CoA + 9FAD_{red} + 9NAD_{red}$	163
18	$CH_3(CH_2)_{16}COCoA + 8FAD_{ox} + 8NAD_{ox} + 8CoA + 8H_2O \rightarrow 9acetyl\text{-}CoA + 8FAD_{red} + 8NAD_{red}$	146
16	$CH_3(CH_2)_{14}COCoA + 7FAD_{ox} + 7NAD_{ox} + 7CoA + 7H_2O \rightarrow 8acetyl\text{-}CoA + 7FAD_{red} + 7NAD_{red}$	129
14	$CH_3(CH_2)_{12}COCoA + 6FAD_{OX} + 6NAD_{OX} + 6CoA + 6H_2O \rightarrow 7acetyl\text{-}CoA + 6FAD_{red} + 6NAD_{red}$	112
12	$CH_3(CH_2)_{10}COCoA + 5FAD_{ox} + 5NAD_{ox} + 5CoA + 5\,H_2O \rightarrow 6acetyl\text{-}CoA + 5FAD_{red} + 5NAD_{red}$	95
10	$CH_3(CH_2)_8COCoA + 4FAD_{ox} + 4NAD_{ox} + 4CoA + 4H_2O \rightarrow 5acetyl\text{-}CoA + 4FAD_{red} + 4NAD_{red}$	78
8	$CH_3(CH_2)_6COCoA + 3FAD_{ox} + 3NAD_{ox} + 3CoA + 3H_2O \rightarrow 4acetyl\text{-}CoA + 3FAD_{red} + 3NAD_{red}$	61
6	$CH_3(CH_2)_4COCoA + 2FAD_{ox} + 2NAD_{ox} + 2CoA + 2H_2O \rightarrow 3acetyl\text{-}CoA + 2FAD_{red} + 2NAD_{red}$	44
4	$CH_3(CH_2)_2COCoA + FAD_{ox} + NAD_{ox} + CoA + H_2O \rightarrow 2acetyl\text{-}CoA + FAD_{red} + NAD_{red}$	27

[a] Total ATP yield per molecule of fatty acyl-CoA ester.

TABLE 5: *β*-Oxidation of Butyryl-CoA to Acetyl-CoA, at 25°C and pH 7.0

Reaction[a]	EC	Reaction	K'	Ref.	$\Delta_r G'^{\circ}_o$/(kJ mol^{-1})
6	1.3.99.2	$CH_3CH_2CH_2COCoA + FAD_{ox} \rightleftharpoons CH_3-CH=CH-COCoA + FAD_{red}$ butyryl-CoA *trans*-2-butenoyl-CoA	41.08	This work	-9.21
7	4.2.1.17	$CH_3-CH=CH-COCoA + H_2O \rightleftharpoons CH_3-CH(OH)-CH_2-COCoA$ *trans*-2-butenoyl-CoA (3*S*)-3-hydroxybutanoyl-CoA	3.45	16	-3.07
8	1.1.1.35	$CH_3-CH(OH)-CH_2-COCoA + NAD_{ox} \rightleftharpoons CH_3-CO-CH_2-COCoA + NAD_{red}$ (3S)-3-hydroxybutanoyl-CoA acetoacetyl-CoA	$6.3\cdot10^{-4}$	17	18.27
9	2.3.1.9	$CH_3COCH_2COCoA + CoA \rightleftharpoons CH_3COCoA + CH_3COCoA$ acetoacetyl-CoA acetyl-CoA acetyl-CoA	$6.41\cdot10^{4}$	18	-27.37
Net reaction:		$CH_3CH_2CH_2COCoA + FAD_{ox} + NAD_{ox} + CoA + H_2O \rightleftharpoons$ butyryl-CoA $CH_3COCoA + CH_3COCoA + FAD_{red} + NAD_{red}$ acetyl-CoA acetyl-CoA	$5.57\cdot10^{3}$	This work	-21.38[a]

[a] 6 = Butyryl-CoA dehydrogenase, EC 1.3.99.2. 7 = Crotonase, Enoyl-CoA hydratase, EC 4.2.1.17. 8 = L-3-Hydroxyacyl-CoA dehydrogenase, EC 1.1.1.35. 9 = Acetyl-CoA C-acetyltransferase, EC 2.3.1.9 $\Delta_r G'^{\circ}$(net) = $\Delta_r G'^{\circ}$ (1) + $\Delta_r G'^{\circ}$ (2) + $\Delta_r G'^{\circ}$ (3) + $\Delta_r G'^{\circ}$ (4).

produce an enoyl-CoA compound (reaction 6). The enoyl-CoA compound is hydrated, producing a hydroxyl compound (reaction 7). The hydroxyl group is then oxidized by NAD_{ox} into the corresponding keto group (reaction 8), and the ensuing carbonyl compound is split by coenzyme A into a fatty acyl-CoA ester, shorter by two carbon atoms, and acetyl-CoA (reaction 9). Reactions 6−9 are analyzed in depth in Table 5.

The calculation of data in Table 5 was a complex task because some standard transformed Gibbs energies of formation and standard transformed Gibbs energies of reaction are not available in the literature. The standard transformed Gibbs energies of formation are not available for *trans*-2-butenoyl-CoA and (3*S*)-3-hydroxybutanoyl-CoA, and the equilibrium constant of reaction was not measured experimentally for the enzyme butyryl-CoA dehydrogenase (EC 1.3.99.2). However, the standard transformed Gibbs energies of formation for all other reactants are known,[19,20] and also the standard transformed Gibbs energies of reaction are known for reactions 7, 8, and 9 in Table 5.[16−18] Therefore, it was possible to calculate all the data in Table 5 by stepwise replacements of unknown values by known values, until all unknowns were eliminated. Horizontally, the sums were checked by the equation

$$\Delta_r G'^{\circ}(\mathrm{pH, I}) = \sum \Delta_f G'^{\circ}_{\mathrm{products}} - \Delta_f G'^{\circ}_{\mathrm{reactants}} \qquad (10)$$

and vertically by summation of $\Delta_r G'^{\circ}$ values.

The standard transformed Gibbs energy of net reaction for degradation of butyryl-CoA by *β*-oxidation to acetyl-CoA, $\Delta_r G'^{\circ}$, was calculated using the equilibrium constants[16−18] obtained under the same conditions. K' for reaction 6 (Table 5) is only an approximate value, and it is not accurate enough for those cases in which a precise value of the equilibrium constant is needed. The enzyme butyryl-CoA dehydrogenase (EC 1.3.99.2) is a flavoprotein oxidoreductase, and hence K' must be determined for reaction in the presence of the enzyme.

Enthalpy and Entropy. Our concepts apply to standard transformed Gibbs energy of formation, but they also apply equally well to standard transformed enthalpy of formation, $\Delta_f H'^{\circ}$, and to standard transformed entropy of formation, $\Delta_f S'^{\circ}$.

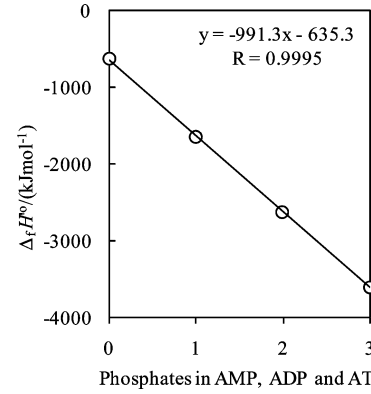

Figure 4. Correlation between the number of phosphates in AMP, ADP, and ATP and the corresponding standard transformed enthalpy of formation, $\Delta_f H'^{\circ}$, in kJ/mol.

Figure 4 displays the correlation between the number of phosphates in AMP, ADP, and ATP and the corresponding standard transformed enthalpy of formation.[8] Figure 5 displays again the correlation between the number of phosphates in AMP, ADP, and ATP and the corresponding standard transformed entropy of formation.[7] Thus, combined, Figures 2, 4, and 5 display the linear correlation of standard transformed Gibbs energy of formation, standard transformed enthalpy of formation, and standard transformed entropy of formation and the number of phosphates in AMP, ADP, and ATP.

The three main thermodynamic functions are connected by the Gibbs relationship

$$\Delta_f G'^{\circ} = \Delta_f H'^{\circ} - T\Delta_f S'^{\circ} \qquad (11)$$

The intercept and slope of the functions in Figures 2, 4, and 5 conform exactly with eq 11.

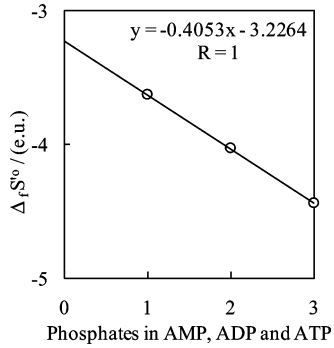

Figure 5. Correlation between the number of phosphates in AMP, ADP, and ATP, and the corresponding standard transformed entropy of formation, $\Delta_f S'^o$, in entropy units.

Conclusion

The primary goal of this communication was to apply the correlation of some thermodynamic properties of structurally similar biological compounds with items such as the number of carbon atoms or the number of its characteristic groups, such as phosphates, to thermodynamics of fatty acid degradation. It was clearly shown that these concepts apply equally well to all three major thermodynamic functions standard transformed: Gibbs energy of formation, enthalpy of formation, and entropy of formation. The second goal was to calculate the $\Delta_f G'^o$ values for major saturated fatty acids, and the third goal was to calculate the $\Delta_r G'^o$ values for enzyme reactions catalyzing complete β-oxidation of butyryl-CoA to acetyl-CoA.

So far, there was little direct experimental evidence for standard transformed Gibbs energies of formation for saturated and unsaturated fatty acids, which have to be determined by direct physical methods. Likewise, there is little experimental evidence for equilibrium constants of enzymatic reactions that catalyze the transformation of fatty acids, which could be determined by the methods of enzyme kinetics.[21,22] Therefore, the further progress in thermodynamics of fatty acid metabolism must await accumulation of novel experimental data.

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References and Notes

(1) Benson, S. W.; Buss, J. H. *J. Chem. Phys.* **1958**, *29*, 546.
(2) Benson, S. W.; Cruickshank, F. R.; Golden, D. M.; Haugen, G. R.; O'Neal, H. E.; Rodgers, A. S.; Shaw, R.; Walsh, R. *Chem. Rev.* **1969**, *69*, 279.
(3) Benson, S. W. *Thermochemical Kinetics*; Wiley&Sons: New York, 1976.
(4) Mavrovouniotis, M. L.; Bayol, P.; Lam, T-K. M.; Stephanopoulos, G.; Stephanopoulos, G. *Biotechnol. Tech.* **1988**, *2*, 23.
(5) Mavrovouniotis, M. L. *Biotechnol. Bioeng.* **1990**, *36*, 1070.
(6) Mavrovouniotis, M. L. *J. Biol. Chem.* **1991**, *266*, 1440.
(7) Alberty, R. A. *Biochemical Thermodynamics*; Wiley: Hoboken, 2006.
(8) Boerio-Goates, J.; Francis, M. R.; Goldberg, R. N.; Ribeiro da Silva, M. A. V.; Ribeiro da Silva, M. D. M. C.; Tewari, Y. B. *J. Chem. Thermodyn.* **2001**, *33*, 929.
(9) Leskovac, V.; Trivić, S.; Peričin, D.; Popović, M.; Kandrač, J. *Ind. J. Biochem. Biophys.* **2008**, *45*, 157.
(10) Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemistry*; W. H. Freeman: New York, 2008.
(11) Streitwieser, A.; Kosower, E. M.; Heathcock, E. M. *Introduction to Organic Chemistry*; Prentice Hall: New York, 1998.
(12) Zubay, G. *Biochemistry*; WCB/McGraw-Hill: Columbus, 1998.
(13) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry*; W. H. Freeman: New York, 2007.
(14) Voet, D., Voet, J. G.; Pratt, C. W. *Fundamentals of Biochemistry: Life at the Molecular Level*; Wiley, John & Sons, 2003.
(15) Goldberg, R. N.; Tewari, Y. B. *J. Phys. Chem. Ref. Data* **1995**, *24*, 1765.
(16) Goldberg, R. N.; Tewari, Y. B. *J. Phys. Chem. Ref. Data* **1995**, *24*, 1669.
(17) Goldberg, R. N.; Tewari, Y. B.; Bell, D.; Fazio, K.; Anderson, E. *J. Phys. Chem. Ref. Data* **1993**, *22*, 515.
(18) Goldberg, R. N.; Tewari, Y. B. *J. Phys. Chem. Ref. Data* **1994**, *23*, 547.
(19) Alberty, R. A. *Thermodynamics of Biochemical Reactions*; Wiley: Hoboken, 2003.
(20) Alberty, R. A. BasicBiochemData; http://www.mathsource.com (accessed 2006).
(21) Leskovac, V. *Comprehensive Enzyme Kinetics*; Kluwer Academic/Plenum Publishers: New York, 2003.
(22) Leskovac, V.; Trivić, S. *Yeast Alcohol Dehydrogenase*; Lambert Academic Publishing: Koeln, 2009.

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