showed no evidence of conversion of porphobilinogen into porphyrins.

5. Porphobilinogen was not found in the liver when rats were killed shortly after its enteral or parenteral administration, nor was it found in the liver of a rat after subcutaneous injections of porphobilinogen every half-hour for 20 hr., during which time a high plasma porphobilinogen concentration was maintained. These findings suggest that the porphobilinogen found in the liver of a rat with experimental porphyria is in fact formed there and is not transported by the blood stream to the liver from an extrahepatic site.

6. The relevance of these results, obtained in the rat, to human acute porphyria, is discussed.

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The Free Energy Change Associated with the Hydrolysis of the Thiol Ester Bond of Acetyl Coenzyme A

By K. BURTON

Department of Biochemistry, University of Chicago and Department of Biochemistry, University of Sheffield

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In previous papers (Burton & Wilson, 1953; Burton & Krebs, 1953), free-energy data were evaluated for the individual reactions of glycolysis, alcoholic fermentation and the tricarboxylic acid cycle. Two approximate values were given for the free energy of hydrolysis of the thiol ester bond of acetyl coenzyme A (acetyl CoA), viz. -13.4 and -11.5 kcal. at pH 7.5.

These estimates were revisions of earlier ones obtained by Stadtman (1952) and by Stern, Ochoa & Lynen (1952) and were based respectively on their equilibrium data for the phosphotransacetylase and the condensing enzyme reactions. Some of the free-energy data used in all these calculations are now known to be seriously in error. Revised calculations described in this article lead to $\Delta G'$ values of

approximately -8 kcal., and these are shown to be consistent with free-energy data for the acetaldehyde-oxidizing system of *Clostridium kluyveri* (Burton, R. M. & Stadtman, 1953).

Revised values are also given for the free energies of formation of the tricarboxylic acids, and the reliability of the previously reported data for the terminal pyrophosphate group of adenosine triphosphate (ATP) is briefly discussed.

Thermodynamic symbols and conventions

These are the same as used previously (Burton & Krebs, 1953). ΔG° is the free energy change for conditions where all the reactants including the H^+ ion are in their thermodynamic standard states. $\Delta G'$ is for conditions where all the reactants except

the H^+ ion are in their standard states. If the H^+ or OH^- ions are not reactants, $\Delta G'$ is the same as ΔG° . The standard state for water is the pure liquid and for carbon dioxide is the gas at 1 atm.

*Free energy of hydrolysis of acetyl CoA
at the thiol ester bond*

Previous calculations based on the equilibrium of the condensing enzyme system (Stern *et al.* 1952; Burton & Krebs, 1953), are now known to contain an error of 5.46 kcal. This is due to an incorrect value for the free energy of formation ($\Delta G^{\circ f}$) of citrate resulting from an arithmetical error in the equilibrium constant of the isocitrate dehydrogenase reaction. This error appeared in Ochoa's (1948) calculation from his experimental results and was pointed out by Harary, Korey & Ochoa (1953).

The revised calculation for the condensing enzyme system using the equilibrium data of Stern *et al.* is shown in the accompanying table. The value of $\Delta G'$ thus obtained for the hydrolysis of acetyl CoA is -7.38 kcal. at pH 7.

Because of this arithmetical error, the previously reported $\Delta G^{\circ f}$ values for citrate, *cis*-aconitate and isocitrate have to be revised and made more negative by 5.46 kcal. The revised $\Delta G^{\circ f}$ values are -279.36, -220.63 and -277.77 kcal., respectively.

It is not certain what value to accept for the $\Delta G^{\circ f}$ of oxalosuccinate. The value previously reported was obtained from the value for isocitrate and from $K = [D\text{-isocitrate}]/[TPN_{ox.}]/[oxalosuccinate]$ $[TPN_{red.}] = 0.3$ at pH 7.2 and 23° (Ochoa, 1948). To be consistent, the $\Delta G^{\circ f}$ value for oxalosuccinate should also be revised by 5.46 kcal. to give -274.2 kcal. However, the corresponding free energy changes for the decarboxylation of oxalosuccinate to α -oxoglutarate and also for the reduction to isocitrate are appreciably different from

the values for the analogous reactions of oxaloacetate.

From Stadtman's (1952) data for the phosphotransacetylase equilibrium and from Korey's unpublished equilibrium data for the acetokinase reaction, Korkes (1952) concluded that ΔG° is practically zero for the reaction:

This means that $\Delta G'$ for the hydrolysis of acetyl CoA is practically the same as that for the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate. After considering the data available for the calculation of the free energy of hydrolysis of ATP, Burton & Krebs (1953) obtained the value of $\Delta G' = -9.4$ kcal. at pH 7.5. This would therefore indicate that $\Delta G'$ for the hydrolysis of acetyl CoA is -9.4 kcal. at pH 7.5 or -8.7 kcal. at pH 7.0. The similar calculations which have been previously reported (Korkes, 1952; Burton & Krebs, 1953) have given numerically larger values either because of using a different value for the hydrolysis of ATP or because an incorrect value was assumed for the acetokinase equilibrium.

From their equilibrium data for the CoA-dependent acetaldehyde-oxidizing system of *Cl. kluyveri*, Burton (R. M.) & Stadtman (1953) obtain two different values for the hydrolysis of acetyl CoA, depending on the ancillary free-energy data used. In this calculation the ancillary data required are the $\Delta G^{\circ f}$ values for aqueous acetaldehyde and the acetate ion together with the free energy of reduction of diphosphopyridine nucleotide (DPN). The data for acetaldehyde and DPN as given by Burton & Wilson (1953) lead to the value of $\Delta G' = -8.25$ kcal., for the hydrolysis of acetyl CoA at pH 7.0. The other set of ancillary data used by Burton (R. M.) & Stadtman gave a value of

Table 1. *Calculation of the free energy of hydrolysis of acetyl CoA*

Reaction A		Reaction B		$\Delta G'$ of hydrolysis of acetyl CoA (kcal.)	
	$\Delta G'$ (kcal.)		$\Delta G'$ (calc.) (kcal.)	(B - A)	(pH 7.0)
Citrate ³⁻ + CoA + H ⁺ ⇌ oxaloacetate ²⁻ + acetyl CoA	7.72 (pH 7.2)	Citrate ³⁻ ⇌ oxaloacetate ²⁻ + acetate ⁻	0.07*	-7.65 (pH 7.2)	-7.38
ATP + acetate ⁻ + CoA ⇌ ADP + acetyl CoA + HPO ₄ ²⁻	0	ATP ⁴⁻ + H ₂ O + H ⁺ ⇌ ADP ³⁻ + HPO ₄ ²⁻	-9.4† (pH 7.5)	-9.4 (pH 7.5)	-8.7
Acetaldehyde + CoA + DPN ⁺ ⇌ acetyl CoA + DPNH + H ⁺	-4.22 (pH 7.0)	Acetaldehyde + DPN ⁺ ⇌ DPNH + acetate ⁻ + H ⁺	-12.47‡ (pH 7.0)	-8.25 (pH 7.0)	-8.25

* Calculated from the $\Delta G^{\circ f}$ values for oxaloacetate and acetate (Burton & Krebs, 1953) and the $\Delta G^{\circ f}$ value for citrate given in the text.

† Burton & Krebs (1953).

‡ Calculated from data for acetaldehyde and the reduction of DPN (Burton & Wilson, 1953) and the $\Delta G^{\circ f}$ value for acetate (Burton & Krebs, 1953).

–13.04 kcal. This value is inaccurate, since the $\Delta G^{\circ}f$ value for acetaldehyde (Parks & Huffman, 1932) is based on an incorrect heat of combustion of ethanol, and also the value (Borsook, 1940) taken for the free energy of reduction of DPN is incorrect.

Of the three values, that of –8.25 kcal. obtained from the data for the acetaldehyde oxidizing system of *Cl. kluyveri* appears to be the most reliable; the most serious uncertainty is that introduced by the data on the heat of combustion of acetic acid. This particular uncertainty is also introduced into the value obtained from the condensing enzyme system (–7.4 kcal.), but it does not apply to the value obtained from the phosphotransacetylase equilibrium and the free energy of hydrolysis of ATP (–8.7 kcal.). Both of these latter calculations are somewhat lengthy, and it is possible that small cumulative errors are introduced at several of the steps.

DISCUSSION

The consistency of the three values obtained for the ΔG° of hydrolysis of the thiol ester bond of acetyl CoA supports the reliability of the free-energy data on which they are based. Two recent independent measurements have confirmed some of the free-energy data which were previously reported. Rodkey (1954) has measured the oxidation–reduction potential of DPN and obtained $E_0' = -0.318$ v at pH 7 and 30°. Equilibrium measurements by Harary *et al.* (1953) for the ‘malic enzyme’ system give a ΔG° value of 0.20 kcal., which is very close to that of 0.18 kcal. which was evaluated indirectly by Burton & Krebs. This agreement confirms the $\Delta G^{\circ}f$ value obtained for pyruvate. It should be noted that the standard state for carbon dioxide in these ΔG° values is gaseous carbon dioxide at 1 atm., whereas Harary *et al.* used 1 M solution as the standard state.

In the data for the steps of glycolysis, there remains an uncertainty in the equilibrium data for the pyruvate–phosphokinase reaction. Utter & Kurahashi (1954) have described the reaction

They report ΔG° to be –9.27 kcal. If, as is likely, the free energy of hydrolysis of the terminal pyrophosphate group of inosine triphosphate (ITP) is the same as that for ATP, this ΔG° value, together with the previously reported $\Delta G^{\circ}f$ values for oxaloacetate, pyruvate and bicarbonate indicate that

ΔG° for the pyruvate–phosphokinase reaction is –15.66 kcal., instead of –14.8 kcal. as previously used. This would give a value of –8.9 kcal. (instead of –9.4 kcal.) for the ΔG° of hydrolysis of ATP to ADP and inorganic phosphate at pH 7.5. At present the 8.9 kcal. value seems to be preferable and this would, with the phosphotransacetylase data be consistent with a ΔG° of –8.2 kcal. (pH 7) for the hydrolysis of acetyl CoA.

SUMMARY

1. Three partially independent calculations are reported for the free energy of hydrolysis of acetyl coenzyme A using free-energy data previously reported by Burton & Krebs (1953). The three calculations are based on the equilibria of (a) the condensing enzyme system, (b) phosphotransacetylase, and (c) the acetaldehyde-oxidizing system of *Clostridium kluyveri*. The values obtained are, at pH 7, $\Delta G^{\circ} = -7.38$, –8.7 and –8.25 kcal. respectively and are considered to be reasonably consistent. At present, a value of about –8.2 kcal. appears to be the most reliable. This is consistent with the phosphotransacetylase data and a slightly revised value of $\Delta G^{\circ} = -8.9$ kcal. for the hydrolysis of the terminal pyrophosphate group of adenosine triphosphate at pH 7.5.

2. Revised values for the free energies of formation ($\Delta G^{\circ}f$) of the citrate³⁻, *cis*-aconitate³⁻ and *D*-isocitrate³⁻ ions are respectively –279.36, –220.63 and –277.77 kcal.

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